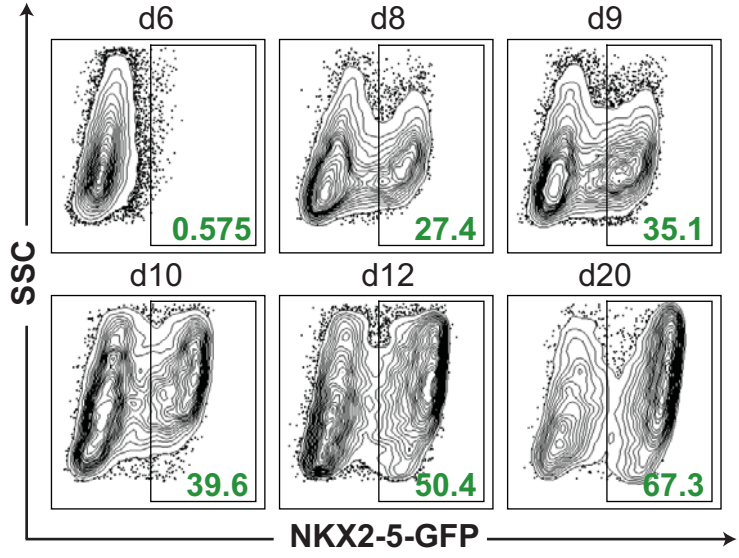
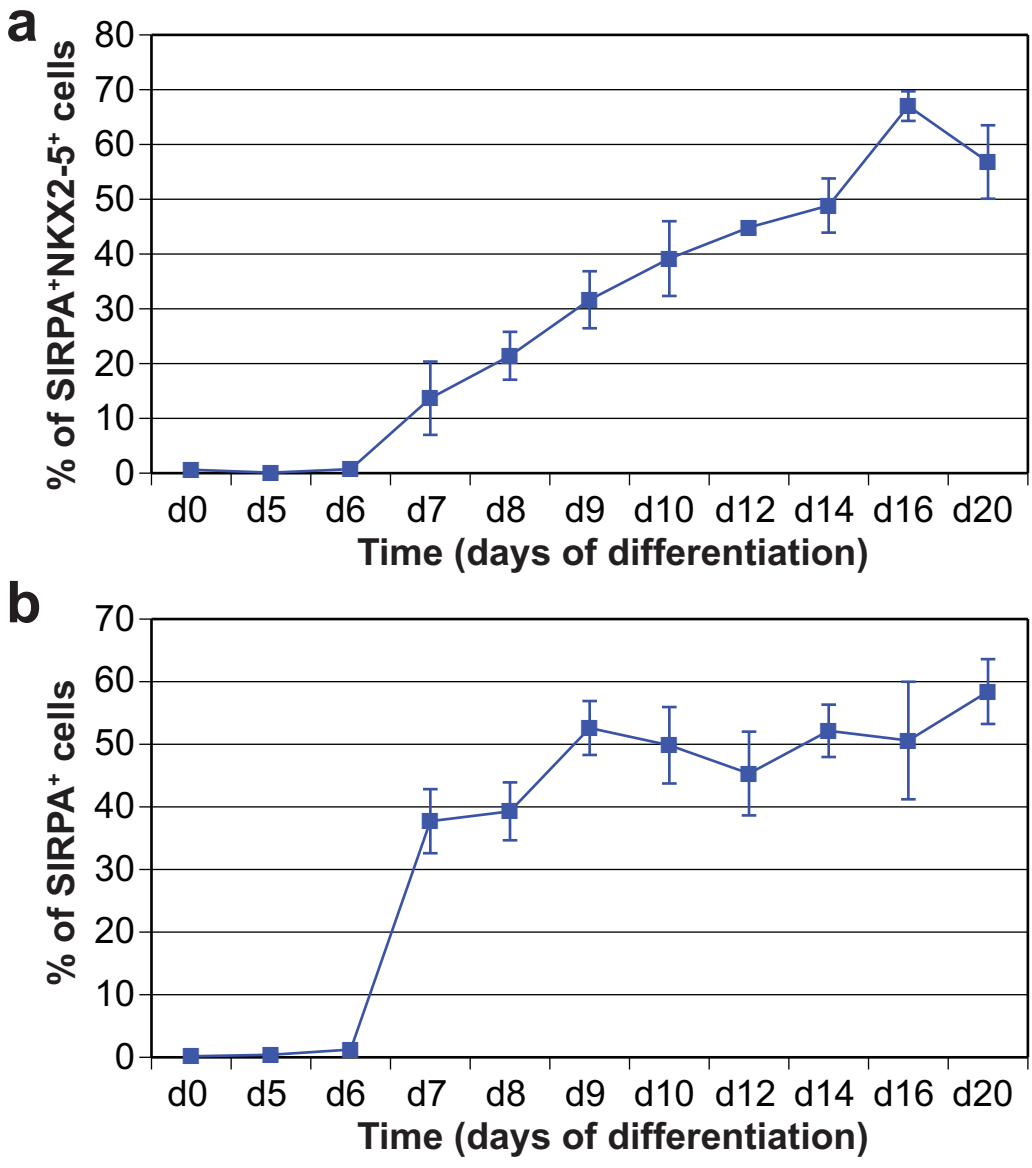
Supplementary Figure 1



Supplementary Figure 1 Differentiation kinetics of the NKX2-5-GFP HES3 hESC line (Elliott et al., 2011). Flow cytometric analysis of EBs derived from the NKX2-5-GFP hESC line at various times during differentiation. GFP expression is first detected at day 8 of differentiation and increases over time with maximum expression at day 20.

Nature Biotechnology: doi:10.1038/nbt.2005

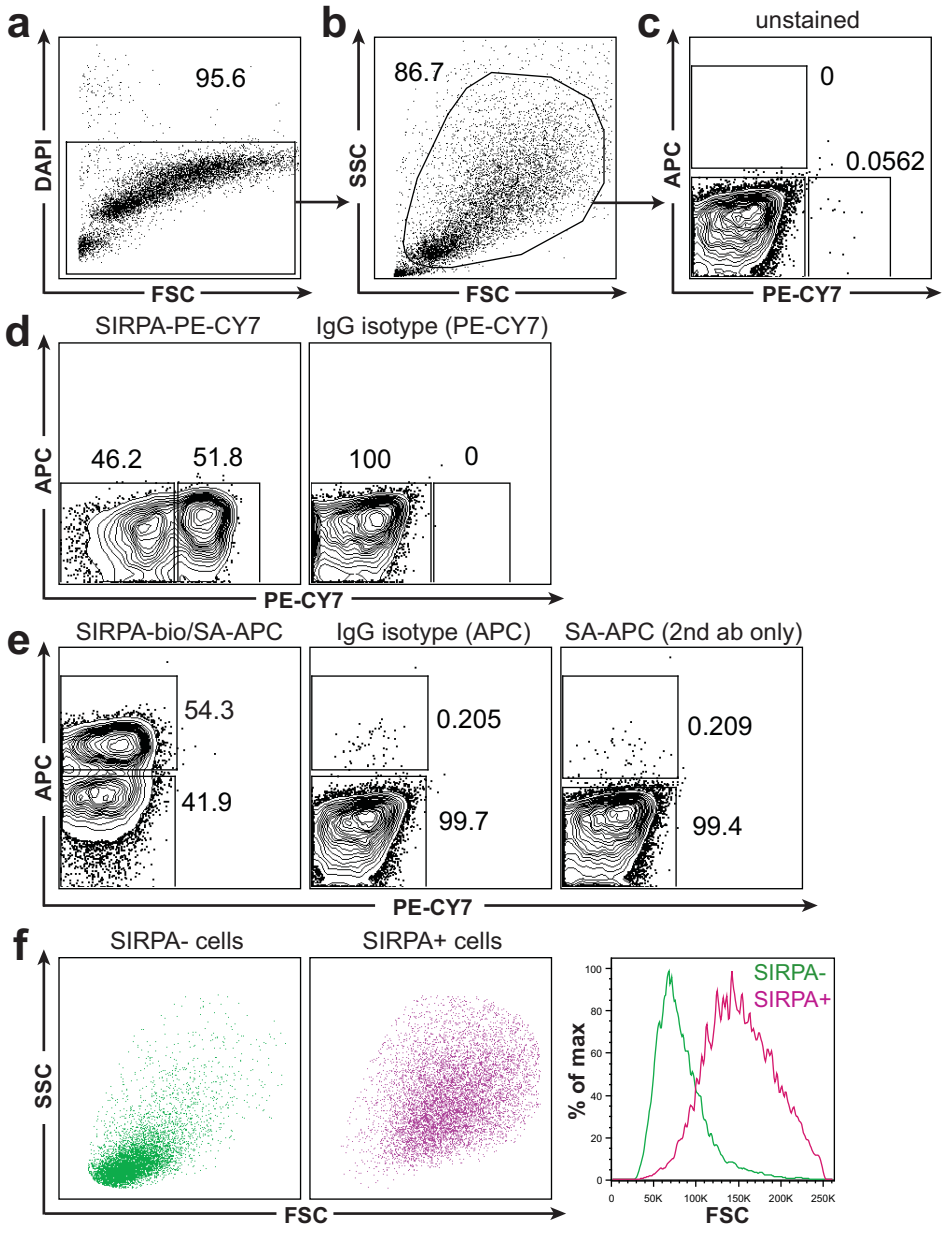
Supplementary Figure 2



Supplementary Figure 2 SIRPA expression kinetics of the NKX2-5-GFP HES3 and the HES2 hESC lines. **(a)** Quantification of SIRPA$^+$/NKX2-5-GFP$^+$ cells by flow cytometric analysis. EBs derived from the NKX2-5-GFP hESC line were analyzed for SIRPA expression at various times during differentiation. Bars represent standard error of the mean, n=5. **(b)** Analysis and quantification of SIRPA$^+$ cells by flow cytometric analysis. EBs derived from the HES2 hESC line were analyzed at various times during differentiation. Bars represent standard error of the mean, n=8. *d0=undifferentiated ES cells, d5-d20=differentiated EBs at day5-day20.*

Nature Biotechnology: doi:10.1038/nbt.2005

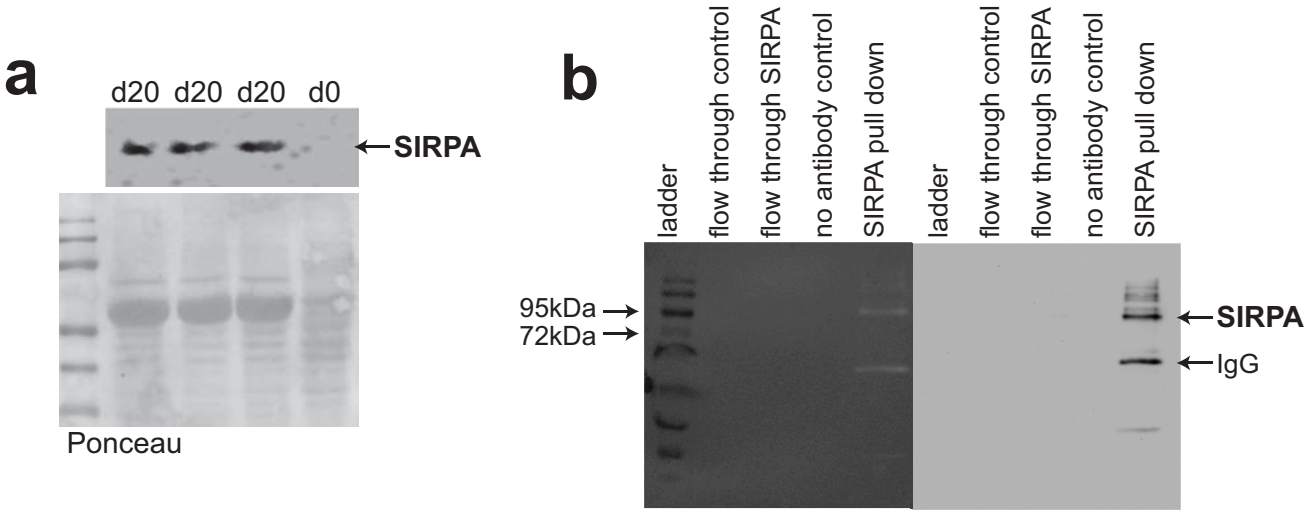
Supplementary Figure 3



Supplementary Figure 3 Flow cytometry analysis strategy and staining controls. **(a)** Flow cytometric analysis of day 20 EB-derived cells (HES2). All cells were stained with the viability dye DAPI and only DAPI-negative cells (=viable cells) were analyzed for each experiment. **(b)** Viable single cells were further defined by FSC/SSC (cell size and granularity) to exclude debris and doublets or cell clumps. **(c)** Unstained control of EB-derived cells at day 20 of differentiation. **(d)** Flow cytometric analysis of day 20 EB-derived cells with the SIRPA-PE-Cy7 antibody and the corresponding IgG control. **(e)** Flow cytometric analysis of day 20 EB-derived cells with the SIRPA-biotin/Streptavidin-APC (SIRPA-bio/SA-APC) antibody combination, the corresponding IgG control and secondary antibody only staining. **(f)** Comparison of cell size between SIRPA- and SIRPA+ cell populations (from (e)) by FSC.

Nature Biotechnology: doi:10.1038/nbt.2005

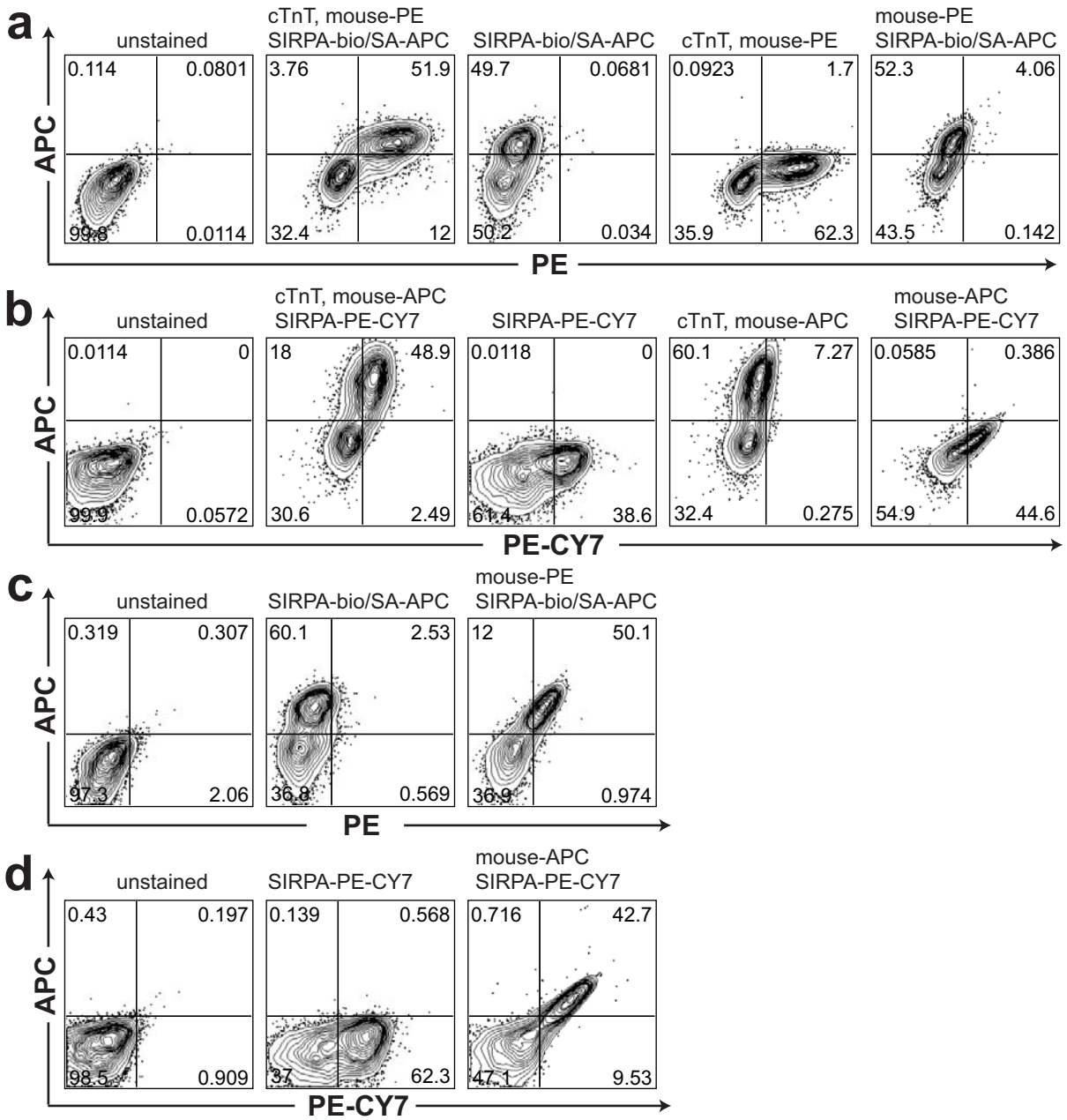
Supplementary Figure 4



Supplementary Figure 4 Western Blot analysis and immunoprecipitation to confirm the specificity of the anti-SIRPA antibody. **(a)** Western Blot analysis of 3 samples from day 20 (d20) differentiation cultures compared to undifferentiated ES cells (d0). The anti-SIRPA SE5A5 antibody was used and Ponceau staining is shown for a loading control. **(b)** Co-immunoprecipitation with the anti-SIRPA SE5A5a antibody with controls. SIRPA runs at the predicted size, as previously described and analyzed in Timms et al., 1999.

Nature Biotechnology: doi:10.1038/nbt.2005

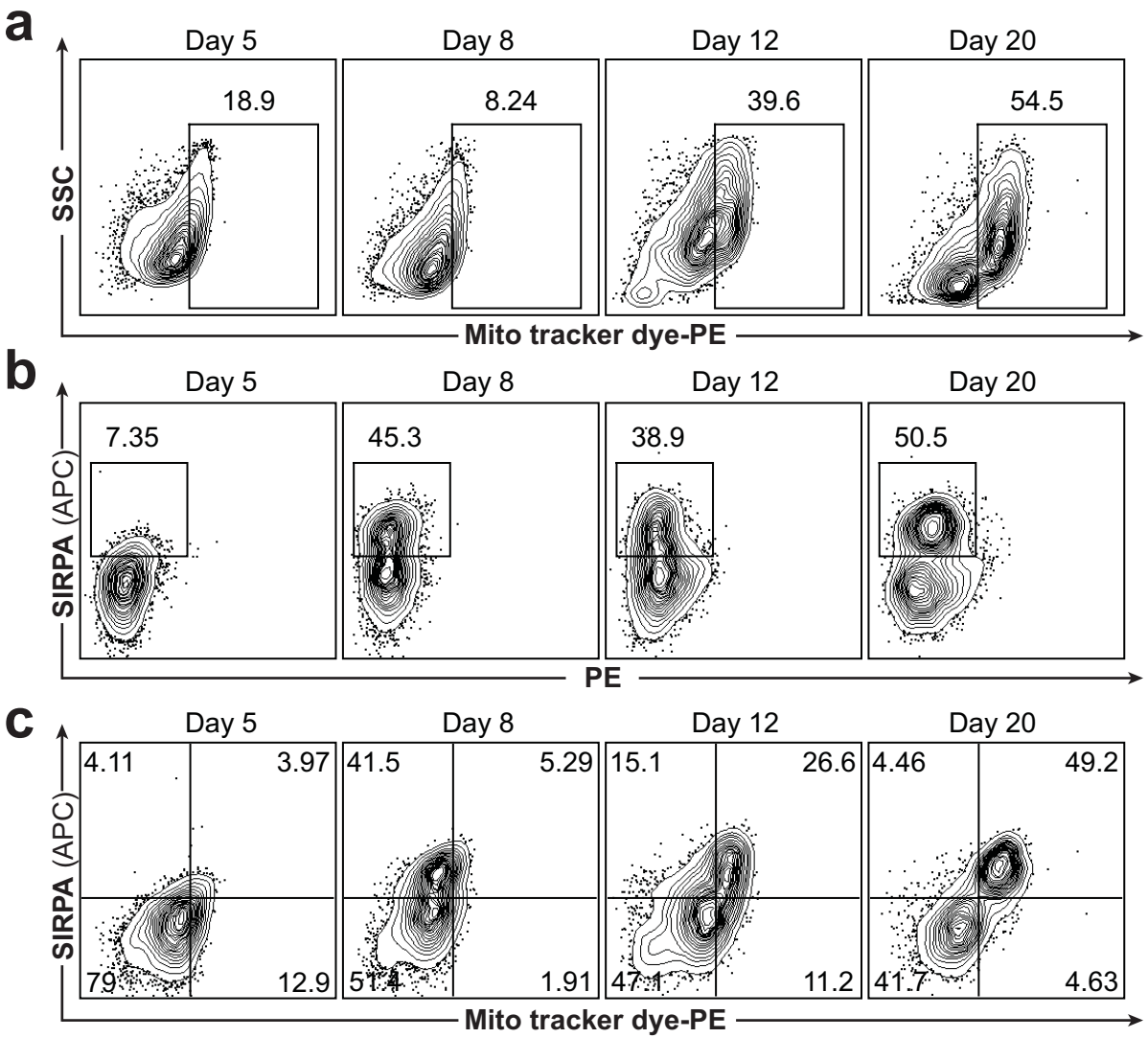
Supplementary Figure 5



Supplementary Figure 5 Co-expression of SIRPA and cTNT by flow cytometry. **(a)** Cells from day 20 HES2-derived EBs were stained for SIRPA first, then fixed (4%PFA, 20min), followed by intracellular staining for cTNT. Since both primary antibodies have been raised in mouse, appropriate controls are shown as well. Cells were stained for anti-SIRPA-biotin/Streptavidin-APC (SIRPA single stain), anti-SIRPA-biotin/Streptavidin-APC and anti-mouse-PE (control to demonstrate that the secondary antibody for cTNT does not recognize SIRPA after fixation), anti-SIRPA-biotin/Streptavidin-APC and anti-cTNT and anti-mouse-PE (SIRPA and cTNT co-staining). **(b)** Same as in (a), but with a different antibody combination. **(c)** Live stain of day20 HES2-derived cells for SIRPA. Co-staining for SIRPA (PE-CY7) and a secondary mouse antibody (APC) demonstrates that, without fixation, mouse secondary antibodies do bind to SIRPA as expected. **(d)** same as in (c) with different antibody combination.

Nature Biotechnology: doi:10.1038/nbt.2005

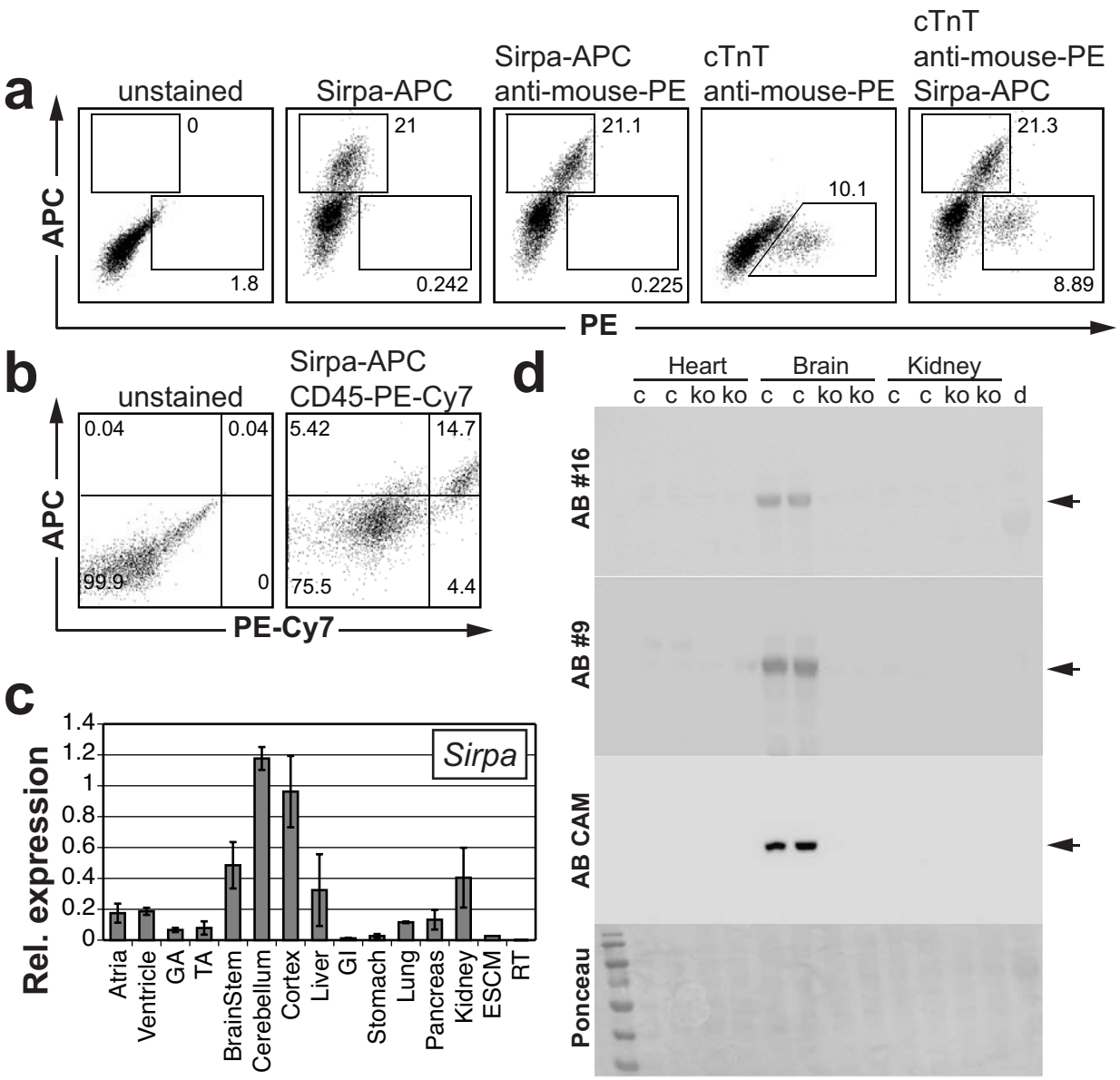
Supplementary Figure 6



Supplementary Figure 6 Comparison of anti-SIRPA antibody staining with mito tracker dye retention labelling. **(a)** Flow cytometric analysis of mito tracker dye labelling at day 5, 8, 12 and 20 of differentiation from HES2 hESCs. **(b)** Flow cytometric analysis of anti-SIRPA at day 5, 8, 12 and 20 of differentiation from HES2 hESCs. **(c)** Co-staining of anti-SIRPA and mito tracker dye labelling followed by flow cytometric analysis at day 5, 8, 12 and 20 of differentiation from HES2 hESCs.

Nature Biotechnology: doi:10.1038/nbt.2005

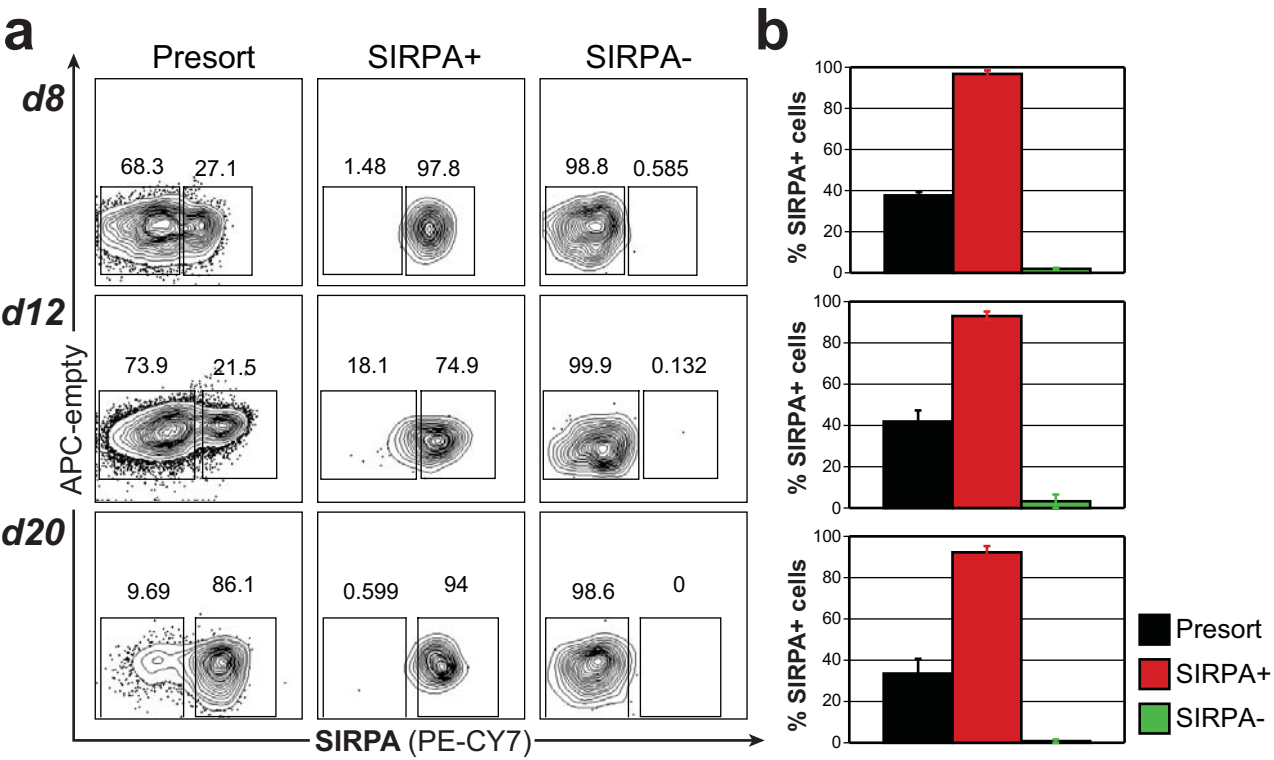
Supplementary Figure 7



Supplementary Figure 7 Analysis of *Sirpa* expression in mouse embryonic stem cell-derived cardiomyocytes and adult mouse tissue samples. **(a)** Flow cytometric analysis of mESC-derived cardiac EB cultures. Cells were stained with Sirpa-APC, fixed with 4%PFA and stained with cTnT/anti-mouse-PE. Sirpa-expressing cells did not co-stain with cTnT-expressing cells, suggesting that cardiomyocytes derived from mES cells do not express Sirpa. **(b)** Flow cytometric analysis of mESC-derived cardiac EB cultures. Sirpa-positive cells co-stain with CD45-PE-Cy7, suggesting that the Sirpa-positive cells present in these cultures represent hematopoietic cells, which have previously been described to express Sirpa (Seiffert et al., 1999). **(c)** RT-qPCR analysis of *Sirpa* in adult mouse tissue samples. *TA,* tibialis anterior muscle; *GA,* gastrocnemius muscle; *GI,* gastrointestinal tract; *RT,* reverse transcriptase control; *ESCM,* mouse embryonic stem cell derived cardiomyocytes from day7 old cultures (Kattman et al., 2011). Mouse brain tissue was used as positive control. Bars represent standard error of the mean, n=4. **(d)** Western blot analysis of adult heart, brain and kidney tissue from control (c) and Sirpa-deficient mice (ko)(Timms et al., 1999) and mouse ESC-derived cardiomyocytes (d). Sirpa expression was solely detected in the brain tissue of control mice, but not in any of the Sirpa-deficient samples or in the control heart, kidney or mESC-derived samples. Antibodies #16 and #9 (specific for cytoplasmic domain, common to all Sirpa isoforms, AB#16, AB#9) were used as described in Timms et al., 1999. ABCAM: anti-Sirpa antibod-

Nature Biotechnology: doi:10.1038/nbt.2005

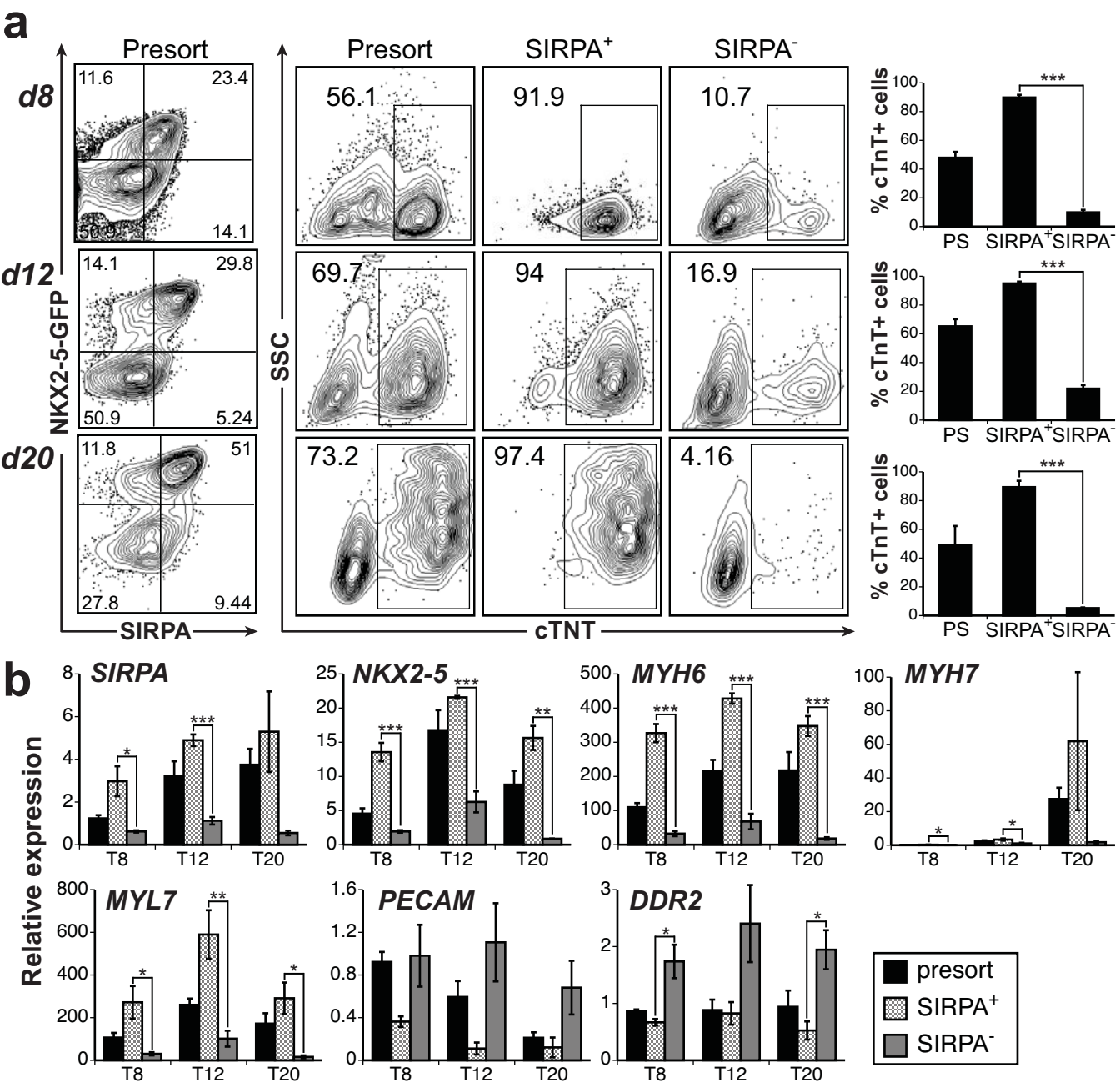
Supplementary Figure 8



Supplementary Figure 8 Analysis of purity of SIRPA- and SIRPA+ fractions following FACS. **(a)** Flow cytometric analysis of presort, SIRPA- and SIRPA+ fraction for SIRPA following cell sorting. **(b)** quantification of SIRPA+ cells in presort, SIRPA- and SIRPA+ fraction following cell sorting. Bars represent standard error of the mean, n=3.

Nature Biotechnology: doi:10.1038/nbt.2005

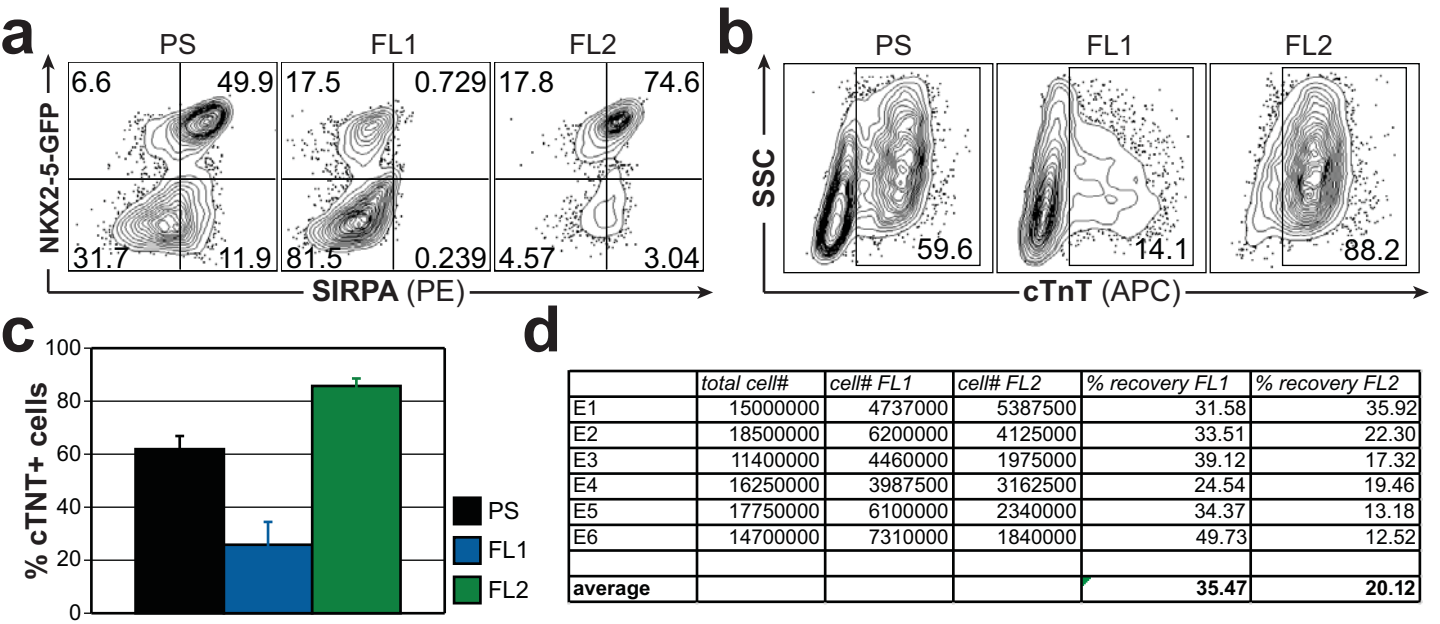
Supplementary Figure 9



Supplementary Figure 9 Enrichment of cardiomyocytes from hESC-derived cultures by cell sorting based on SIRPA expression. **(a)** Flow cytometric analysis of SIRPA expression at day (d)8, d12 and d20 of differentiation from NKX2-5-GFP HES3 hESCs. Fluorescent-activated cell sorting (FACS) for SIRPA was performed at d8, d12 and d20 and the presort (PS), SIRPA$^+$ and SIRPA$^-$ fractions were analysed for cardiac TroponinT (cTnT) expression by intracellular flow cytometry. The frequency of cTnT+ cells at d8, d12 and d20 was significantly higher in the SIRPA$^+$ fraction (day8: 89.8% ± 1.9, day12: 95.0 ± 1.3, day20: 89.4 ± 4.4), compared to SIRPA$^-$ cells (day8: 9.9 ± 1.7, day12: 21.9 ± 2.5, day20: 5.2 ± 0.5), n=3. **(b)** QPCR analysis of PS, SIRPA$^+$ and SIRPA$^-$ cells after cell sorting. Expression of markers specific for the cardiac lineage (*NKX2-5*, *MYH6*, *MYH7* and *MYL7*) was significantly higher in the SIRPA$^+$ compared to SIRPA$^-$ fraction at all stages analyzed (d8, d12 and d20). Expression of markers for the non-cardiac lineages (*PECAM* and *DDR2*) segregated to the SIRPA$^-$ fraction and the PS cells. Bars represent standard error of the mean, n=3.

Nature Biotechnology: doi:10.1038/nbt.2005

Supplementary Figure 10

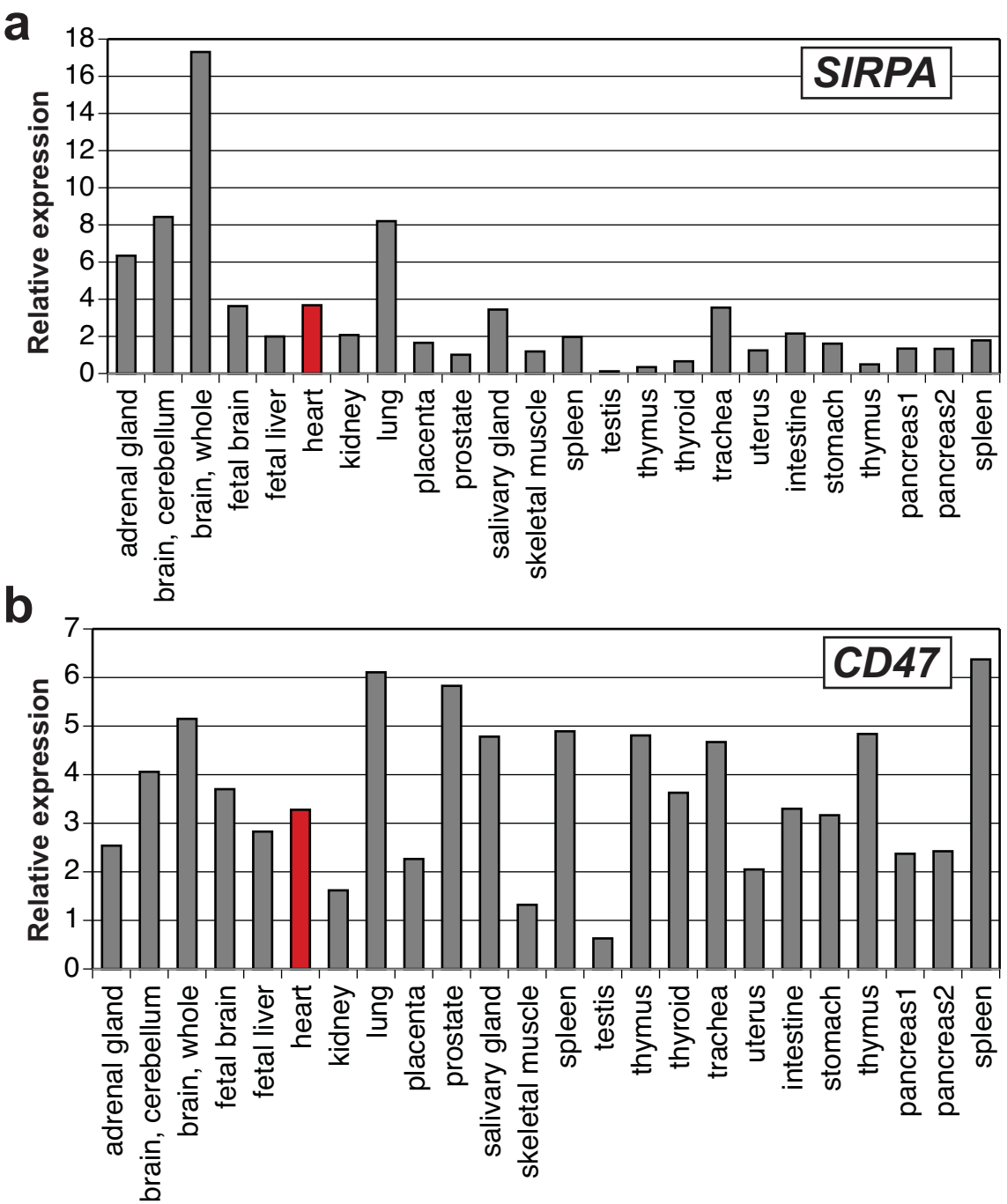


	total cell#	*cell# FL1*	*cell# FL2*	*% recovery FL1*	*% recovery FL2*
E1	15000000	4737000	5387500	31.58	35.92
E2	18500000	6200000	4125000	33.51	22.30
E3	11400000	4460000	1975000	39.12	17.32
E4	16250000	3987500	3162500	24.54	19.46
E5	17750000	6100000	2340000	34.37	13.18
E6	14700000	7310000	1840000	49.73	12.52
average				**35.47**	**20.12**

Supplementary Figure 10 Isolation of SIRPA$^+$ cardiomyocytes via bead sorting. **(a)** Flow cytometric analysis of SIRPA. NKX2-5GFP-derived EBs were sorted using the Miltenyi magnetic bead sorting system. *In brief:* cells were labelled with SIRPA-PE antibody (20min) and incubated with anti-PE beads (15min, 4C). The labelled cells were passed through a positive selection column (LS colums). Flow through 1 (FL1, SIRPA-depleted fraction) and flow through 2 (FL2, SIRPA-enriched fraction, eluted from the column) were collected and analyzed. PS, FL1 and FL2 fractions after sorting were analyzed for SIRPA expression. **(b)** Intracellular cTnT flow cytometric analysis of PS, FL1 and FL2 fractions. **(c)** Quantification of cTNT-expression of the PS, FL1 and FL2 fractions. **(d)** Quantification of the efficiency of magnetic bead sorting.

Nature Biotechnology: doi:10.1038/nbt.2005

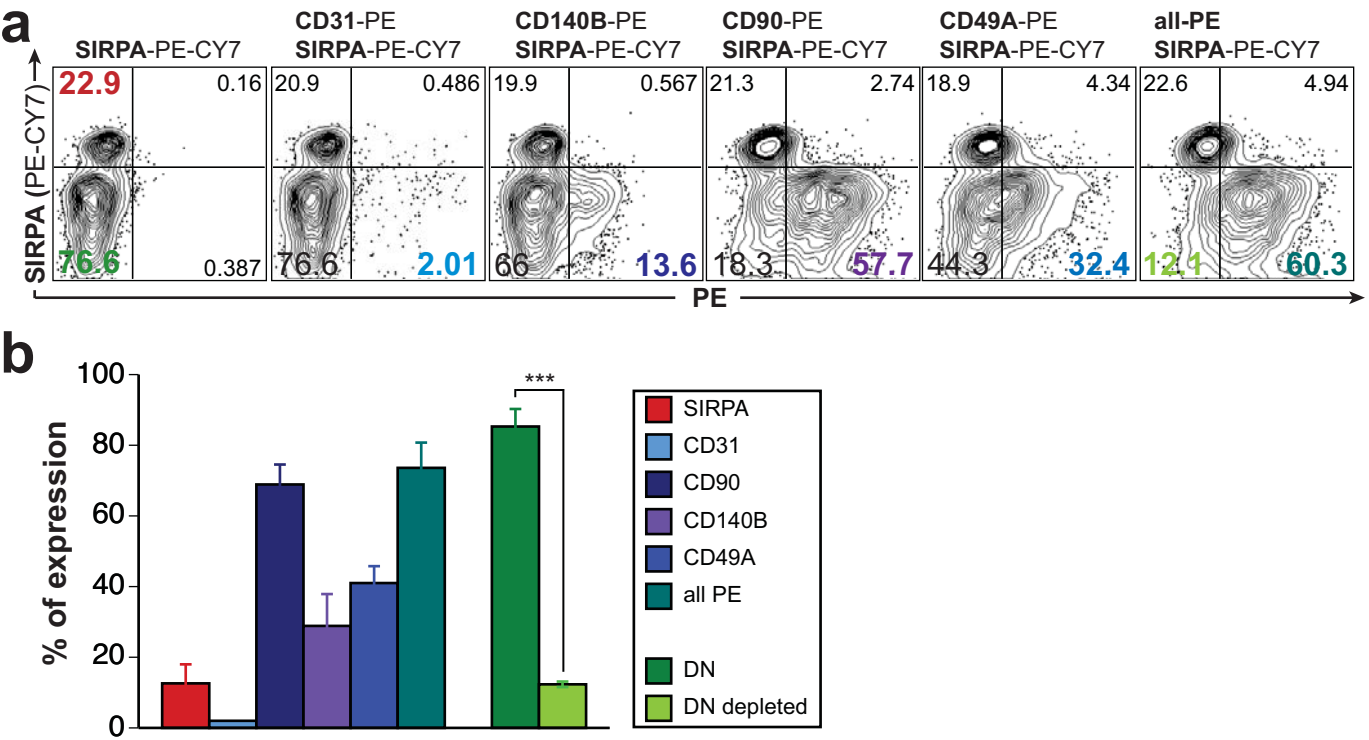
Supplementary Figure 11



Supplementary Figure 11 Gene expression analysis of human adult tissue. **(a)** RT-qPCR analysis of SIRPA. **(b)** RT-qPCR analysis of CD47.

Nature Biotechnology: doi:10.1038/nbt.2005

Supplementary Figure 12



Supplementary Figure 12 Expression of non-myocyte markers from the iPSC line MSC-iPS1-derived differentiation cultures. **(a)** Flow cytometric analysis of markers specifically expressed on non-myocyte (SIRPA-) cells in day 20 differentiation cultures. **(b)** Quantification of expression of non-myocyte markers by flow cytoemtry at day 20 of differentiation from MSC-iPS1 iPS cells. *DN*, all SIRPA-negative cells (76.6% in (a), first panel); *DN depleted*, remaining duble negative cells after staining with SIRPA and the all-PE lineage cocktail (12.1% in (a), last panel).

Nature Biotechnology: doi:10.1038/nbt.2005

Supplementary Table 1

a

	Total cell#	SIRPA- cell#	SIRPA- (%)	SIRPA- exp cell#	Eff SIRPA-	Eff SIRPA - (%)
E1	5920000	844000	31.28	1851776	0.46	**45.58**
E2	3070000	594670	32.98	1012486	0.59	**58.74**
E3	2200000	372940	33.57	738540	0.50	**50.50**
E4	6980000	1380000	34.08	2378784	0.58	**58.01**
E5	34900000	4700000	20.42	7126580	0.66	**65.95**
E6	14200000	2590000	50.5	7171000	0.36	**36.12**
E7	17100000	3650000	36.12	6176520	0.59	**59.09**
E8	15390000	476000	5.24	806436	0.59	**59.03**
Average						**54.13**

b

	Total cell#	SIRPA+ cell#	SIRPA+ (%)	SIRPA+ exp cell#	Eff SIRPA+	Eff SIRPA+ (%)
E1	5920000	419000	31.3	1852960	0.23	**22.61**
E2	3070000	163830	10.17	312219	0.52	**52.47**
E3	2200000	93300	8.56	188320	0.50	**49.54**
E4	6980000	461000	30.08	2099584	0.22	**21.96**
E5	34900000	1640000	17.79	6208710	0.26	**26.41**
E6	14200000	299000	10.8	1533600	0.19	**19.50**
E7	17100000	1930000	32.98	5639580	0.34	**34.22**
E8	15390000	4650000	65.15	10026585	0.46	**46.38**
E9	14800000	3600000	55.23	8174040	0.44	**44.04**
Average						**35.24**

Supplementary Table 1 Efficiency of fluorescent-activated cell sorting (FACS) with the anti-SIRPA antibody. **(a)** Recovery of SIRPA$^-$ cells after FACS of EB-derived cells from HES2 at day20 of differentiation, n=8. **(b)** Recovery of SIRPA$^+$ cells after FACS of EB-derived cells from HES2 at day20 of differentiation, n=9. *Total cell #* = total cells passed through the flow cytometer; *SIRPA$^-$(SIRPA$^+$)#* = total *SIRPA$^-$*(SIRPA$^+$) cells recovered after the sorting procedure; *SIRPA$^-$(SIRPA$^+$)%* = percentage of SIRPA$^-$(SIRPA$^+$) cells determined by staining with the SIRPA antibody; *SIRPA$^-$(SIRPA$^+$) exp cell#* = cell number of SIRPA$^-$(SIRPA$^+$) cells expected based on staining with the SIRPA antibody and on total cell number sorted; *Eff SIRPA$^-$(SIRPA$^+$)* = efficiency of SIRPA$^-$(SIRPA$^+$) cell recovery: SIRPA$^-$(SIRPA$^+$) cell# / SIRPA$^-$(SIRPA$^+$) exp cell#; *Eff SIRPA$^-$(SIRPA$^+$)* = efficiency of SIRPA$^-$(SIRPA$^+$) cell recovery in percentage.

Supplementary Table 2

a

total cell#	LIN- cell#	LIN- (%)	LIN- exp cell#	Eff LIN-	Eff LIN- (%)
10800000	2770000	78.65	8494200	0.32	**32.61**
5850000	531100	21.73	1271205	0.42	**41.78**
14000000	1110000	18.12	2536800	0.44	**43.76**
26000000	2350000	26.73	6949800	0.34	**33.81**
15080000	3430000	51.31	7737548	0.44	**44.33**
10300000	3000000	47.89	4932670	0.60	**60.82**
average					**42.85**

b

total cell#	LIN+ cell#	LIN+ (%)	LIN+ exp cell#	Eff LIN+	Eff LIN+ (%)
10800000	680000	10.32	1114560	0.61	**61.01**
5850000	157700	5.48	320580	0.49	**49.19**
14000000	1360000	20.42	2858800	0.48	**47.57**
26000000	3810000	38.55	10023000	0.38	**38.01**
15080000	1960000	21.37	3222596	0.61	**60.82**
10300000	2200000	35.9	3697700	0.59	**59.50**
average					**52.68**

Supplementary Table 2 Efficiency of fluorescent-activated cell sorting (FACS) with the non-myocyte markers CD31, CD90, CD140B and CD49A (LIN). **(a)** Recovery of LIN^- cells after FACS of EB-derived cells from HES2 at day20 of differentiation, n=6. **(b)** Recovery of LIN^+ cells after FACS of EB-derived cells from HES2 at day20 of differentiation, n=6. *Total cell #* = total cells passed through the flow cytometer; *$LIN^-(LIN^+)$#* = total $LIN^-(LIN^+)$ cells recovered after the sorting procedure; *$LIN^-(LIN^+)$%* = percentage of $LIN^-(LIN^+)$ cells determined by staining with the LIN antibodies; *$LIN^-(LIN^+)$ exp cell#* = cells number of $LIN^-(LIN^+)$ cells expected based on staining with the LIN antibodies and on total cell number sorted; *Eff $LIN^-(LIN^+)$* = efficiency of $LIN^-(LIN^+)$ cell recovery: $LIN^-(LIN^+)$ cell# / $LIN^-(LIN^+)$ exp cell#; *Eff $LIN^-(LIN^+)$* = efficiency of $LIN^-(LIN^+)$ cell recovery in percentage.

Nature Biotechnology: doi:10.1038/nbt.2005

Supplementary Table 3

GENE	FORWARD 5′-3′	REVERSE 5′-3′
hTBP	TGAGTTGCTCATACCGTGCTGCTA	CCCTCAAACCAACTTGTCAACAGC
hBRACHURY (T)	TGTCCCAGGTGGCTTACAGATGAA	GGTGTGCCAAAGTTGCCAATACAC
hMESP1	AGCCCAAGTGACAAGGGACAACT	AAGGAACCACTTCGAAGGTGCTGA
hISL1	GAAGGTGGAGCTGCATTGGTTTGA	TAAACCAGCTACAGGACAGGCCAA
hNKX2-5	TTTGCATTCACTCCTGCGGAGACCTA	ACTCATTGCACGCTGCATAATCGC
hMYH6	TCAGCTGGAGGCCAAAGTAAAGGA	TTCTTGAGCTCTGAGCACTCGTCT
hMYH7	TCGTGCCTGATGACAAACAGGAGT	ATACTCGGTCTCGGCAGTGACTTT
hMYL2	TGTCCCTACCTTGTCTGTTAGCCA	ATTGGAACATGGCCTCTGGATGGA
hMYL7	ACATCATCACCCATGGAGACGAGA	GCAACAGAGTTTATTGAGGTGCCC
hFOXA2	GCATTCCCAATCTTGACACGGTGA	GCCCTTGCAGCCAGAATACACATT
hNEUROD	TCCCATGTCTTCCACGTTAAGCCT	CATCAAAGGAAGGGCTGGTGCAAT
hPECAM	TTCCTGACAGTGTCTTGAGTGGGT	TTTGGCTAGGCGTGGTTCTCATCT
hPDGFRB	TGGGCTAGACACGGGAGAATACTT	AAGATGTAGAGCCGTTTCCGCTCA
hDDR2	ACCAGCCATTTGTCCTGACTCTGT	ATCACTCGTCGCCTTGTTGAAGGA
hTHY1	ATACCAGCAGTTCACCCATCCAGT	ATTTGCTGGTGAAGTTGGTTCGGG
hSIRPA	ACCTGGCTCAGGCTAGTTCCAAAT	TGTGCACACGTATGTGCTGTCTCT
hCD47	AGCTCTAAACAAGTCCACTGTCCC	TCCTGTGTGTGAGACAGCATCACT

Supplementary Table 3 Primer sequences used for RT qPCR analysis

Nature Biotechnology: doi:10.1038/nbt.2005