SUPPORTING INFORMATION

Knockdown of μ-calpain in Fanconi Anemia, FA-A, cells by siRNA Restores αII Spectrin levels and Corrects Chromosomal Instability and Defective DNA Interstrand Cross-link Repair

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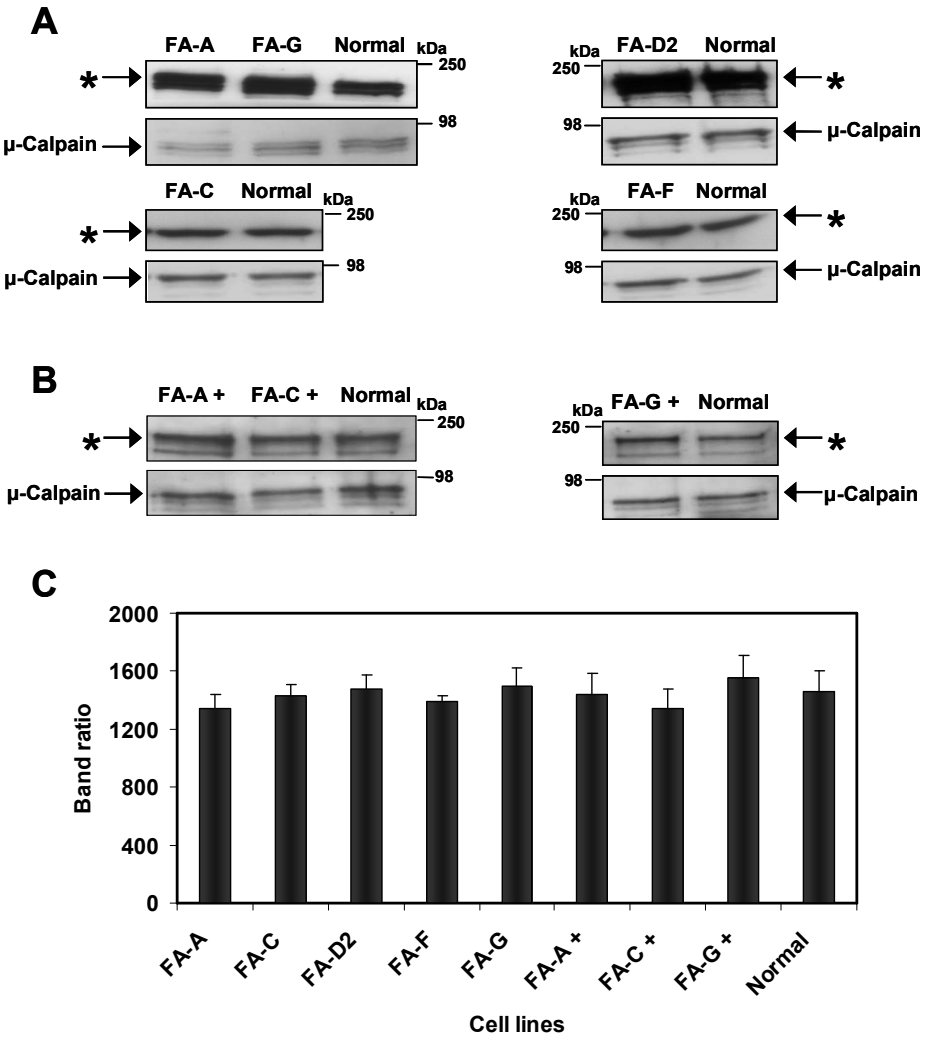

FIGURE S1. Levels of μ-calpain protein in FA cells are the same as those in corrected FA cells and normal cells. Chromatin-associated protein extracts from (A) FA-A, FA-G, FA-C, FA-D2, and FA-F and (B) corrected FA-A+, FA-C+ and FA-G+ cells were examined for levels of μ-calpain using western blot analysis and compared with μ-calpain levels found in these extracts from normal cells. Blots were probed with anti-μ-calpain. Topoisomerase (*) was used as a loading control. Molecular weight markers are as indicated. (C) These immunoblots were scanned and levels of μ-calpain quantitated. Vertical lines represent $\pm$ s.e.m. for 4-6 experiments.

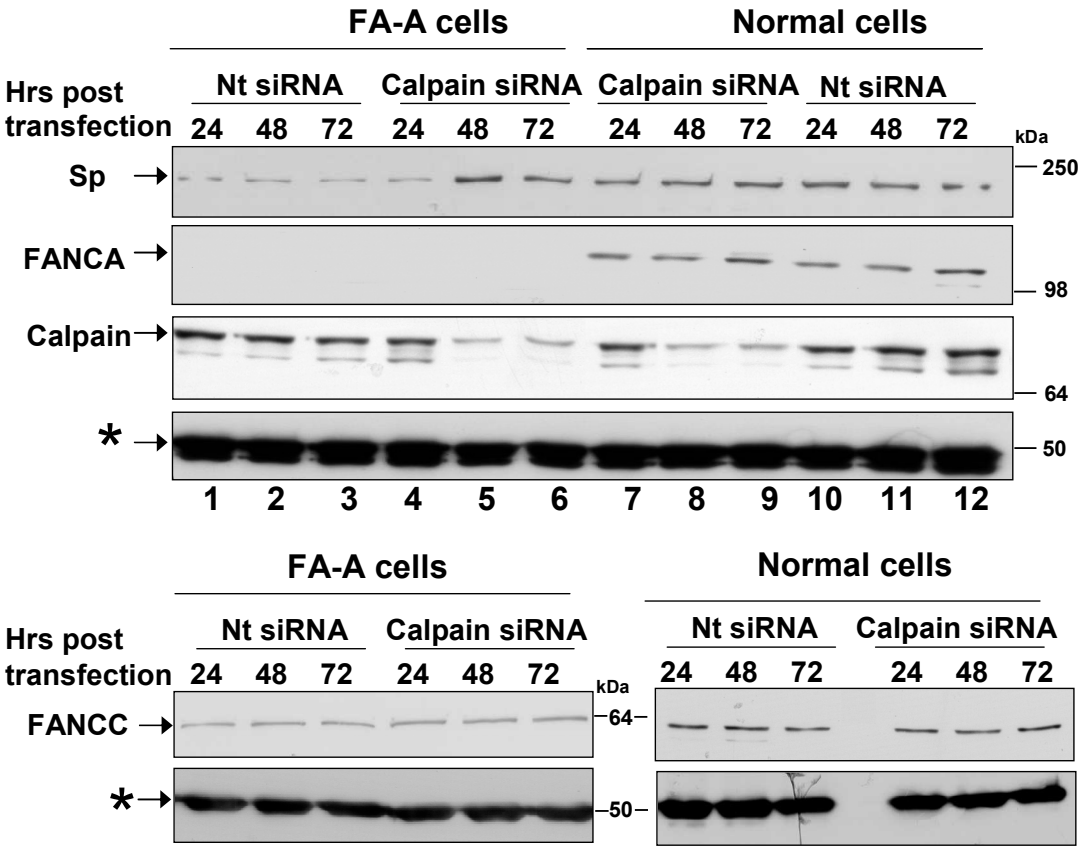

FIGURE S2. Knocking down μ-calpain in FA-A cells by siRNA leads to restoration of levels of αIISp to normal 48 hours post transfection and has no effect on levels of FANCA or FANCC. FA-A and normal cells were transiently transfected with 300 pM μ-calpain siRNA or nontarget (Nt) siRNA. Levels of αIISp, μ-calpain, FANCA and FANCC in the FA-A and normal cells were examined by western blot analysis at 24, 48 and 72 hours post transfection. Immunoblots were probed with anti-αIISp, anti-μ-calpain, anti-FANCA, and anti-FANCC antibodies. Tubulin (*) was used as a loading control.

Table S1. MMC-induced chromosomal aberrations in normal cells transfected with nontarget (Nt) siRNA and FA-A cells transfected with Nt siRNA or μ-calpain siRNA

	MMC (30 nM)	Cells with chromosomal aberrations[a]	Chromosomal aberrations[b]			
			Interchromatid exchanges	Fusions/ radials	Breaks	Total chromosomal aberrations[b]
Normal +	-	12	9	6	3	18
Nt siRNA	+	34	65	28	39	132
FA-A	-	14	15	5	5	25
	+	95	401	97	130	628
FA-A +	-	16	10	4	7	21
Nt siRNA	+	92	393	87	118	598
FA-A +	-	17	18	2	4	24
μ-calpain siRNA	+	48	80	11	55	146

[a] 100 cells in each group were counted to determine how many cells contained metaphase spreads with chromosomal aberrations (i.e. interchromatid exchanges, fusions/radials and breaks)

[b] Chromosomal aberrations found in metaphase spreads from 100 cells in each group