Supplementary information for:

REDD1 is essential for stress-induced synaptic loss and depressive behavior

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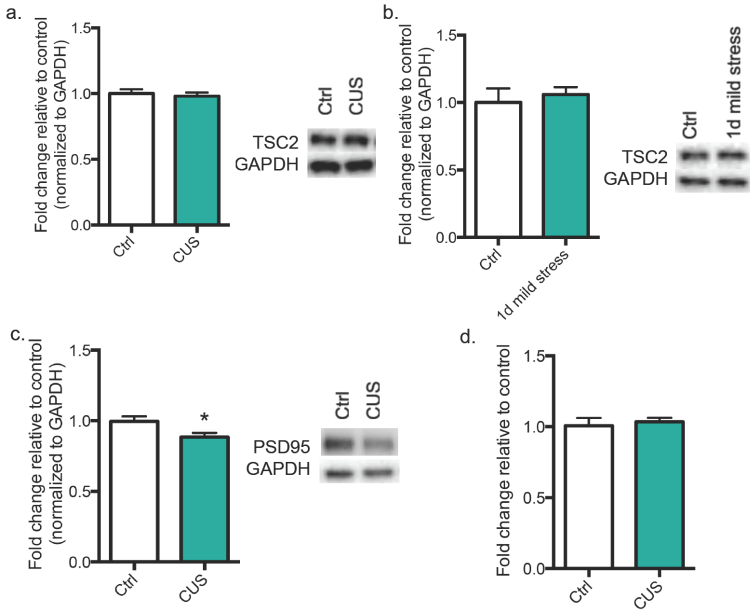
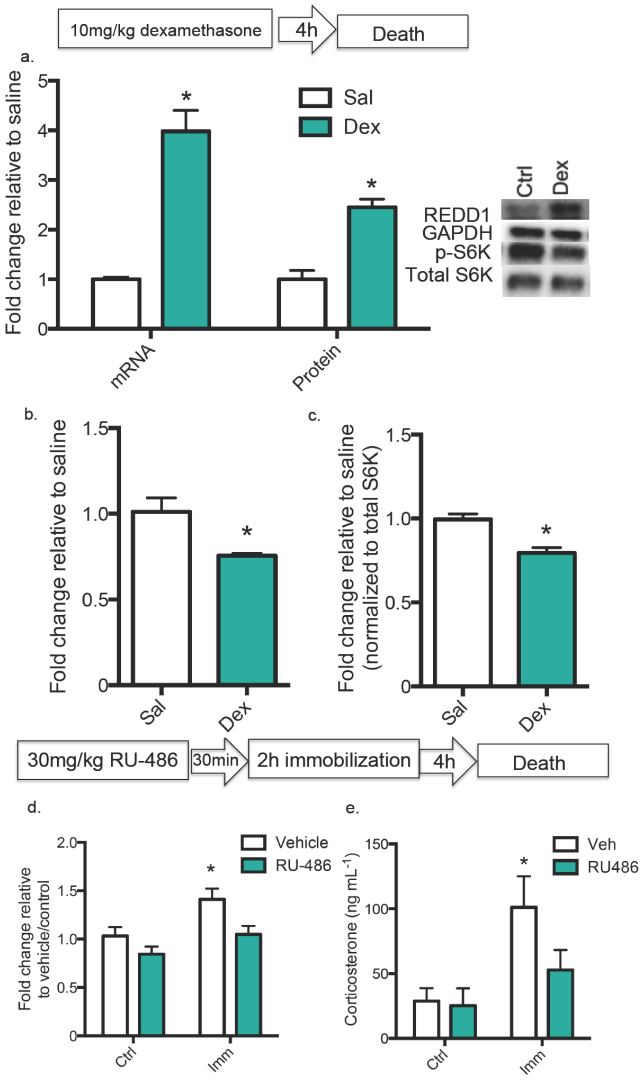
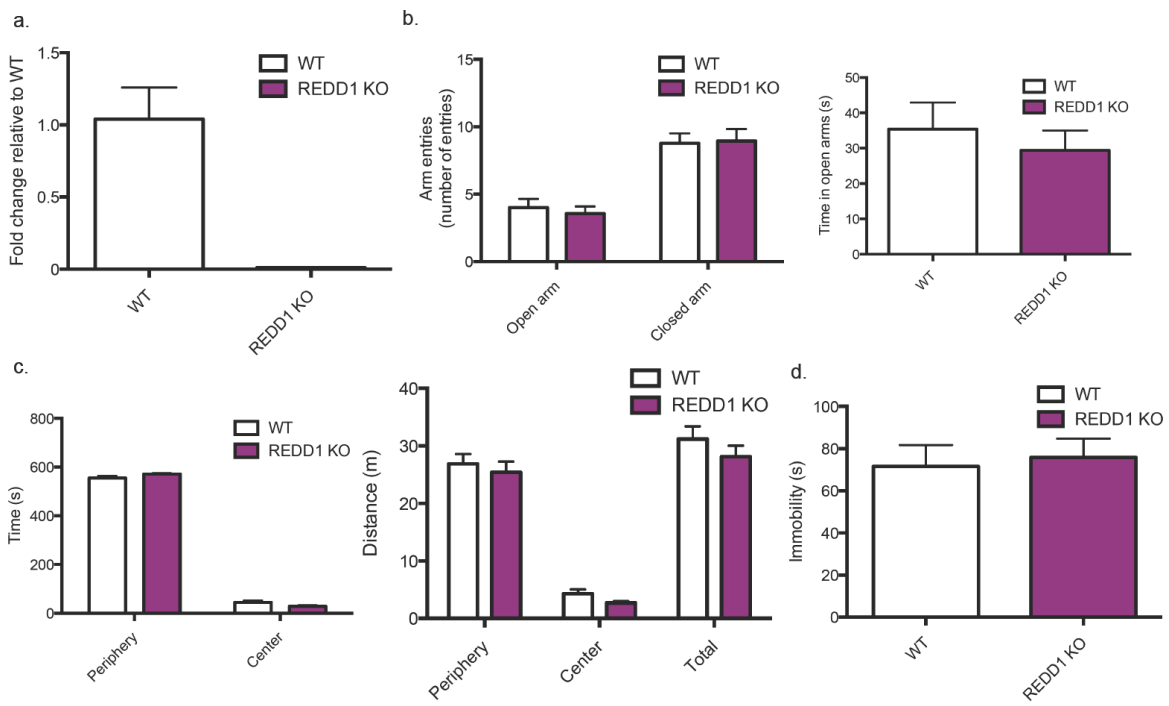


Supplementary Figure 1. Regulation of the TSC2/REDD1/protein synthesis signaling pathway by CUS. (**a**) Rats were subjected to 21 d of CUS (*n*=10) or regular handling (control) (*n*=12) then sacrificed 4 h following the final stressor. Mean (± SEM) fold change for TSC2 protein from PFC dissections. Here, protein levels have been normalized to GAPDH. Representative blots for TSC2 and GAPDH are displayed to the right. (**b**) Rats were subjected to 1 d of mild stress (*n*=6) or handling (control) (*n*=6) then sacrificed 4 h following the final stressor. Mean (± SEM) fold change for TSC2 protein from PFC dissections. Here, protein levels have been normalized to GAPDH. Representative blots for TSC2 and GAPDH are displayed to the right. (**c**) Rats were subjected to 21 d of CUS (*n*=14) or regular handling (control) (*n*=14) then sacrificed 4 h following the final stressor. Mean (± SEM) fold change for PSD-95 protein from PFC dissections. Here, protein levels have been normalized to GAPDH. Representative blots for PSD-95 and GAPDH are displayed to the right. (**d**) Rats received 21 d of CUS (*n*=8) or regular handling (control) (*n*=8) then sacrificed 4 h following the final stressor. Results are mean ± SEM fold change of hippocampal REDD1 mRNA relative to control. (*) $p < 0.05$ relative to control.



Supplementary Figure 2. Glucocorticoids regulate REDD1 expression in the PFC. (**a**) Rats were given one 10 mg kg^{-1} injection of dexamethasone (Dex) then sacrificed 4 h following the final stressor. Control animals were injected with saline (Sal). Mean (± SEM) fold change in REDD1 mRNA and protein from PFC dissections relative to control for either Dex (*n*=6) or Sal (*n*=6) rats. Here, protein levels have been normalized to GAPDH. Representative blots for REDD1 and GAPDH are displayed to the right. (**b**) Results are mean ± SEM fold change of PFC REDD2 mRNA [Dex *n*=5; Sal *n*=6] relative to Sal. (**c**) Mean (± SEM) fold change in phospho-S6K PFC dissections for either Dex (*n*=12) or Sal (*n*=12) rats. Here, protein levels have been normalized to

total S6K. Representative blots for p-S6K and total S6K are displayed above. (**d**) Rats were given one 30 mg kg^{-1} injection of RU-486, immobilized 30 min later for 2 h, then sacrificed 4 h later. Results are mean ± SEM fold change of PFC REDD1 mRNA [Veh/Ctrl n=8; RU/Ctrl n=6; Veh/Imm n=10; RU/Imm n=8] relative to Veh/Ctrl. (**e**) Mean ± SEM serum corticosterone in ng mL^{-1} [Veh/Ctrl n=8; RU/Ctrl n=5; Veh/Imm n=9; RU/Imm n=7]. (*) $p < 0.05$ relative to control.



Supplementary Figure 3. REDD1 knock out mice show no baseline behavioral alterations. (**a**) Mean ± SEM fold change (0.01 ± 0.002) PFC REDD1 mRNA [WT *n*=3; REDD1 KO *n*=4] relative to control. (**b**) *Left*. Mean number of entries (± SEM) into the open and closed arms of the elevated plus maze is depicted for WT (*n*=14) and REDD1 KO (*n*=19) mice. *Right*. Mean time (± SEM) in open arms of the elevated plus maze is depicted for WT (*n*=14) and REDD1 KO (*n*=19) mice. (**c**) *Left*. Mean time (± SEM) in periphery and center of open field box is depicted for WT (*n*=14) and REDD1 KO (*n*=19) mice. *Right*. Mean distance traveled (± SEM) in the periphery, center, and total, of the open field box is depicted for WT (*n*=14) and REDD1 KO (*n*=19) mice. (**d**) Mean number of seconds immobile (± SEM) during the forced swim test is depicted for WT (*n*=30) and REDD1 KO (*n*=36) mice.

Group	Age (yr)	Gender	PMI (h)	pH	Cause of death (suicide/other)	Medications
Control	48.45±1.84	22M/16F	18.97±0.84	6.66±0.04	0/38	n.d.
MDD	47.89±1.87	18M/20F	18.32±0.87	6.56±0.04	12/26	21

Supplementary Table 1. Summary of demographic information for all postmortem subjects. Case demographics of MDD subjects. Abbreviations: PMI, post-mortem interval; M, male; F, female; n.d., no psychotropic medication detected; MDD, major depressive disorder.

a. Cohort 1 Control

	Age	Gender	PMI (Hours)	Tissue pH	Psychotropic Drugs	Suicide
	60	M	10	6.8	n.d.	N
	22	M	20	7.0	n.d.	N
	64	F	20	6.7	n.d.	N
	51	M	16	6.5	n.d.	N
	58	F	23	6.4	n.d.	N
	39	F	25	6.8	n.d.	N
	55	F	11	6.8	n.d.	N
	47	M	17	6.6	n.d.	N
	51	F	8	6.6	n.d.	N
	52	F	23	7.1	n.d.	N
	23	F	9	6.1	n.d.	N
	54	M	21	6.3	n.d.	N
	46	F	15	6.8	n.d.	N
	37	F	24	6.7	n.d.	N
	43	M	22	6.6	n.d.	N
	40	F	17	6.8	n.d.	N
	56	M	16	6.8	n.d.	N
	45	F	12	6.7	n.d.	N
	46	M	22	6.3	n.d.	N
	58	F	19	6.7	n.d.	N
	65	F	19	6.6	n.d.	N
	53	M	23	6.8	n.d.	N
	56	M	23	6.5	n.d.	N
	57	M	16	6.2	n.d.	N
	36	F	15	6.4	n.d.	N
	45	M	17	6.6	n.d.	N
	37	M	21	6.6	n.d.	N
Avg	**48.00**		**17.81**	**6.62**		
SEM	*2.11*		*0.92*	*0.05*		

Cohort 1 MDD

	Age	Gender	PMI (Hours)	Tissue pH	Psychotropic drugs	Suicide
	57	M	13	7.1	n.d.	Y
	24	M	13	6.9	n.d.	Y
	65	F	18	7.0	Y	N
	47	M	11	6.8	n.d.	N
	54	F	18	6.2	Y	N
	40	F	22	6.6	n.d.	N
	53	F	12	6.7	Y	N
	46	M	16	6.3	n.d.	Y
	52	F	10	6.5	Y	N
	49	F	23	6.4	Y	N
	26	F	13	6.4	Y	Y
	57	M	16	6.6	Y	N
	47	F	22	6.6	n.d.	Y
	28	F	25	6.6	n.d.	N
	44	M	19	6.5	n.d.	N
	40	F	11	6.5	Y	N
	58	M	13	6.8	Y	N
	39	F	13	6.4	Y	Y
	46	M	23	6.6	Y	N
	50	F	19	6.4	Y	N
	64	F	12	6.6	Y	N
	51	M	25	6.5	n.d.	N
	55	M	24	6.5	n.d.	N
	61	M	16	6.6	n.d.	N
	37	F	16	6.6	Y	N
	42	M	14	6.4	Y	Y
	36	M	20	6.8	Y	N
Avg	**46.96**		**16.91**	**6.57**		
SEM	*2.10*		*0.92*	*0.04*		

b. Cohort 2 Control

	Age	Gender	PMI (Hours)	Tissue pH	Psychotropic Drugs	Suicide
	38	M	30	6.7	n.d	N
	33	M	23	6.9	n.d.	N
	54	M	17	6.9	n.d.	N
	50	F	27	6.7	n.d.	N
	69	M	18	6.7	n.d.	N
	54	M	26	6.5	n.d.	N
	32	M	25	6.9	n.d.	N
	39	M	21	6.7	n.d.	N
	60	F	19	6.4	n.d.	N
	66	M	12	7.2	n.d.	N
	50	M	22	6.8	n.d.	N
Avg	**49.55**		**21.82**	**6.76**		
SEM	*3.84*		*1.55*	*0.06*		

Cohort 2 MDD

	Age	Gender	PMI (Hours)	Tissue pH	Psychotropic drugs	Suicide
	33	M	18	6.8	Y	N
	36	M	11	7.0	n.d.	N
	54	M	23	6.2	Y	N
	50	F	23	6.8	Y	Y
	73	F	17	6.6	Y	N
	52	M	17	6.5	n.d.	Y
	34	F	24	6.3	n.d.	Y
	42	F	24	6.6	n.d.	Y
	63	F	24	6.3	Y	N
	65	M	30	6.2	n.d.	Y
	50	F	28	6.5	n.d.	N
Avg	**50.18**		**21.75**	**6.53**		
SEM	*3.99*		*1.66*	*0.08*		

Supplementary Table 2. Complete demographic information for postmortem subjects. (**a**) Demographic information (age, gender, PMI, tissue pH, psychotropic drugs, and suicide) for cohort 1 subjects. (**b**) Demographic information (age, gender, PMI, tissue pH, psychotropic drugs, and suicide) for cohort 2 subjects.