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Supplemental information

TRAF7 is an essential regulator of blood vessel integrity during mouse embryonic and neonatal development

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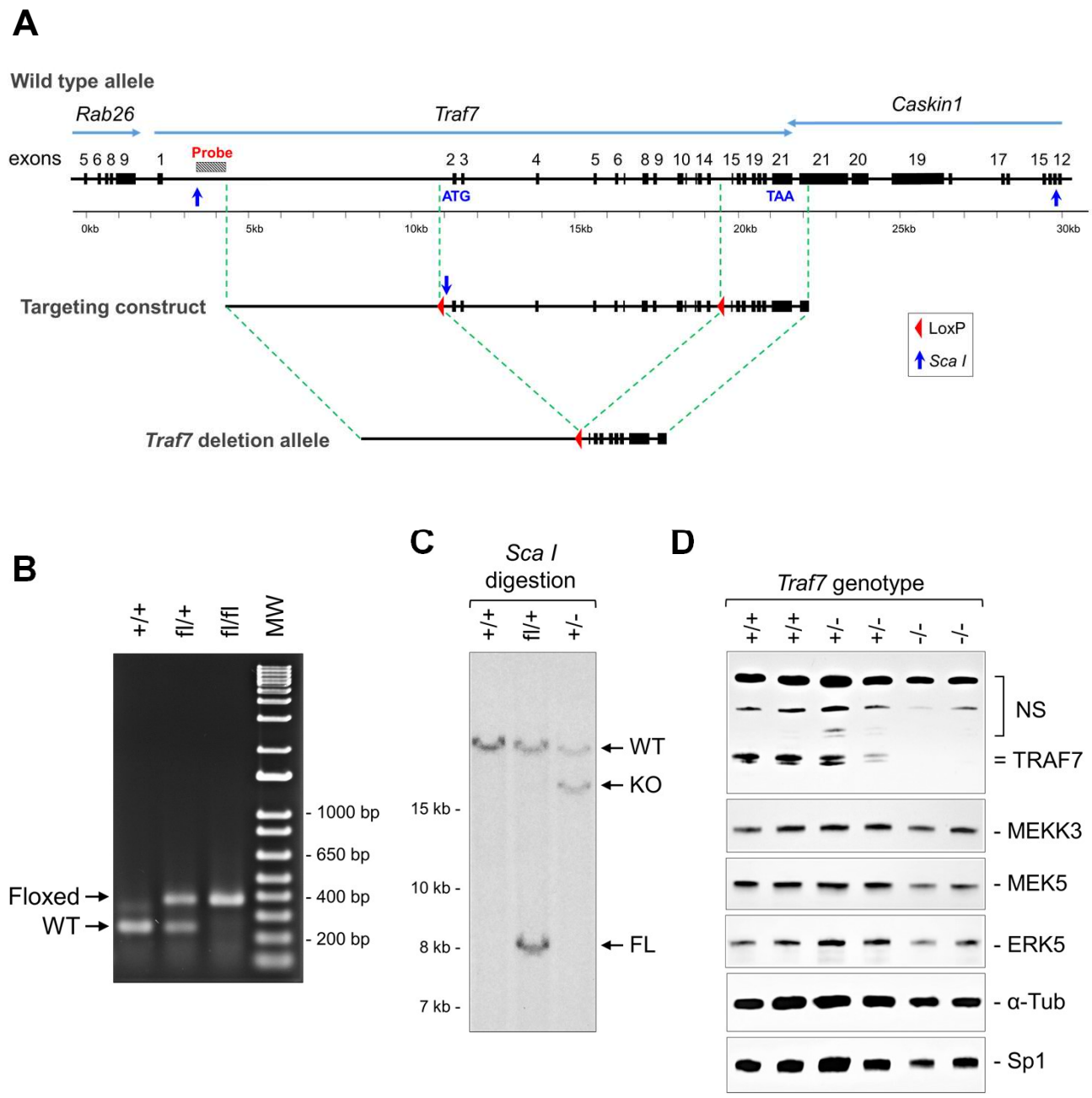


Figure S1. Targeted deletion of Traf7 gene.
(**A**) Schematic drawing (to-scale) of the *Traf7* gene and part of the surrounding locus on mouse chromosome 17. DNA is shown as a black line. Exons are represented as black boxes and numbered. Directions of gene transcription are indicated by blue arrows above. LoxP sites are shown as red triangles. *Sca I* restriction sites used in Southern blot analysis are marked by blue arrows. Maps of "Targeting construct" and the resulting "*Traf7* deletion allele" are positioned below the "Wild type allele" with corresponding dotted green lines illustrating the Cre-mediated *Traf7* gene excision process. (**B**) Detection of WT and *Traf7*-floxed alleles by conventional PCR and agarose gel electrophoresis. (MV): Molecular Weight markers (1 Kb Plus Ladder). (**C**) Southern blot analysis of genomic DNA purified from WT (+/+), $Traf7^{fl/+}$ (fl/+), and $Traf7^{GLOhet}$ (+/-) mouse tails, digested with *Sca I*, and probed with region spanning the genomic region from intron 1 of the *Traf7* gene immediately outside of the targeting construct as shown in red font in (A). (**D**) Western blot analysis of TRAF7, MEKK3, MEK5, and ERK5 protein expression in WT (+/+), $Traf7^{GLOhet}$ (+/-), and $Traf7^{GLOko}$ (-/-) E9.5 whole embryo lysates. Two different embryos of each genotype were processed independently. Anti-α-Tubulin and anti-Sp1 antibodies were used as loading controls. (NS): non-specific bands. Related to Figure 1.

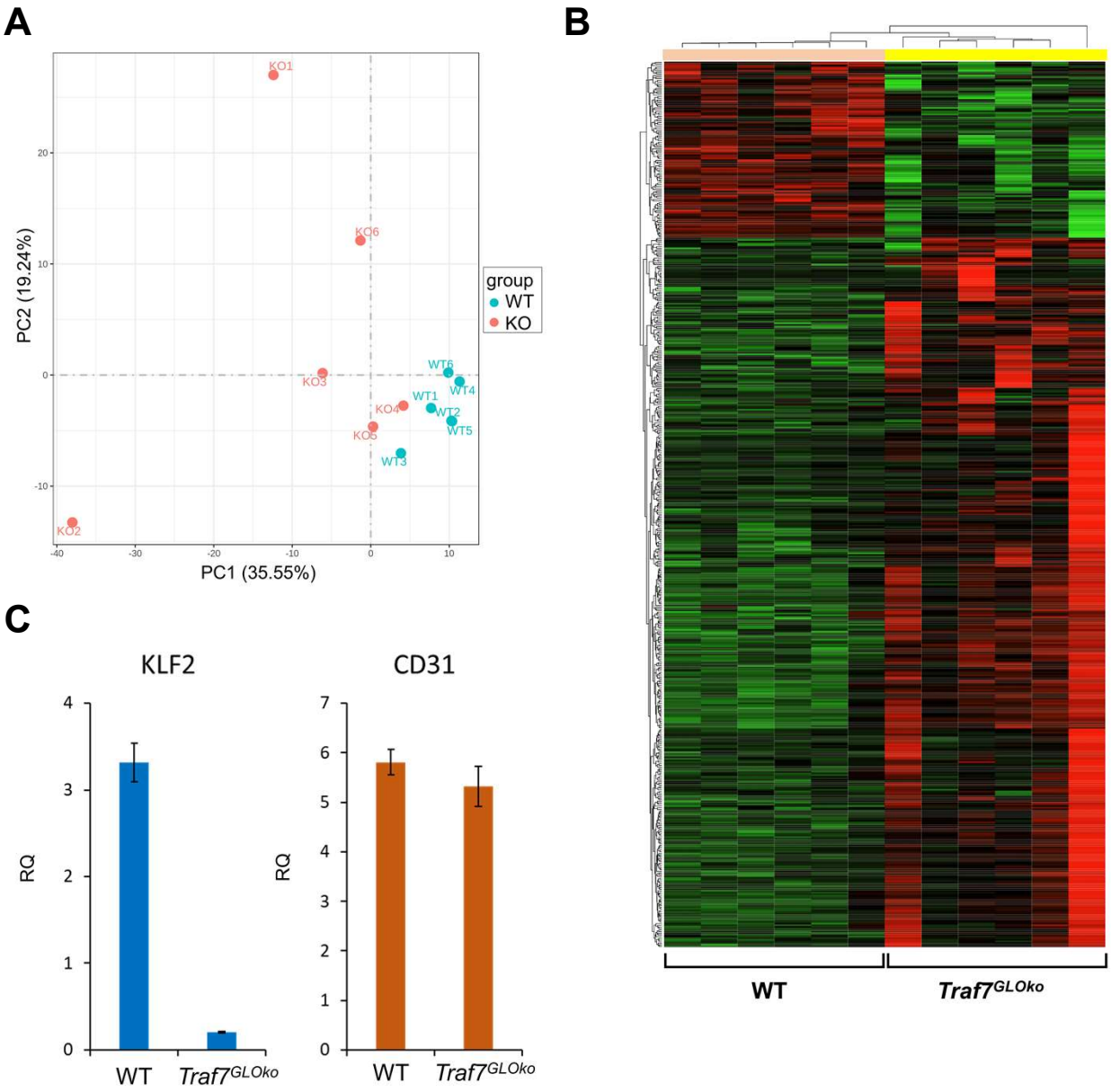


Figure S2. Differential gene expression in WT and *Traf7*-deficient mouse embryos.
(**A**) PCA Plot of RNA-seq analysis in WT and *Traf7*^{GLOko} (KO) embryos at E9.5. Each point corresponds to an individual embryo. (**B**) Heatmap of mRNA expression levels for all significant DEGs in WT versus *Traf7*^{GLOko} embryos by RNA-seq. (**C**) mRNA expression analysis of *Klf2* and *Cd31* in WT (n=6) versus *Traf7*^{GLOko} (n=8) embryos at E9.5. Total RNA was purified from whole mouse embryos and mRNA was amplified by RT-qPCR using *Gapdh* as internal control. Data are presented as relative quantity (RQ) Mean ± SEM. Related to Figure 3.

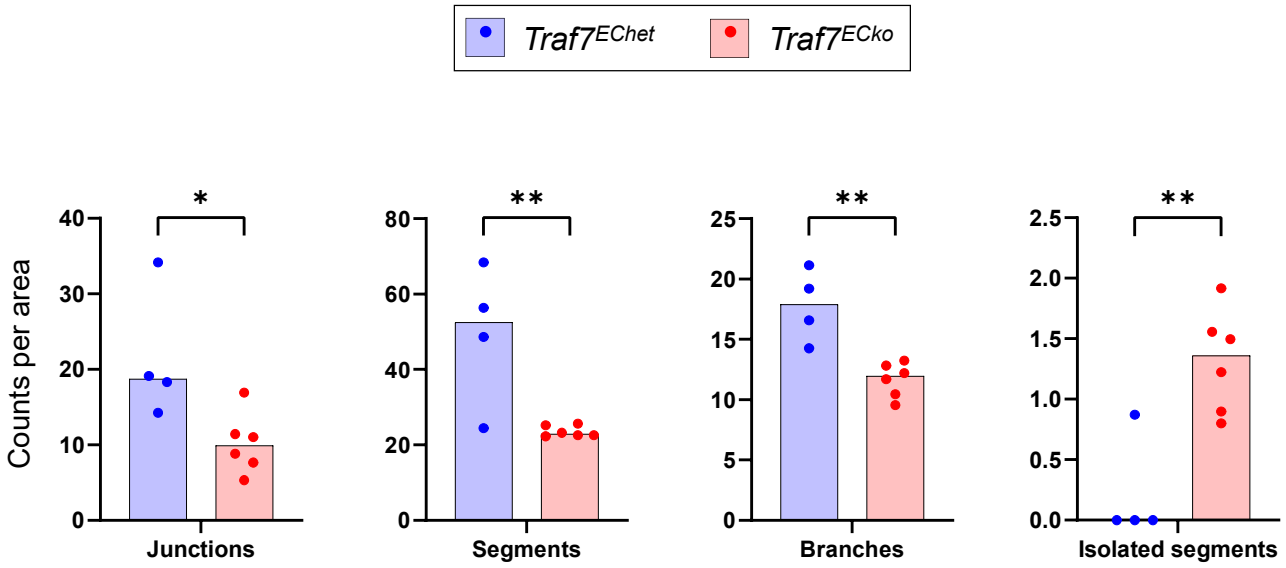


Figure S3. Quantification of the embryonic vascular defect in *Traf7*-deficient mouse embryos. Vascular network analysis was performed using Angiogenesis Analyzer in ImageJ software. Number of branching points (junctions), segments, and branches were analyzed within a fixed-size window in at least four different embryos of each genotype and plotted as the Median. (*) $p < 0.05$; (**) $p < 0.005$. Related to Figure 4C.

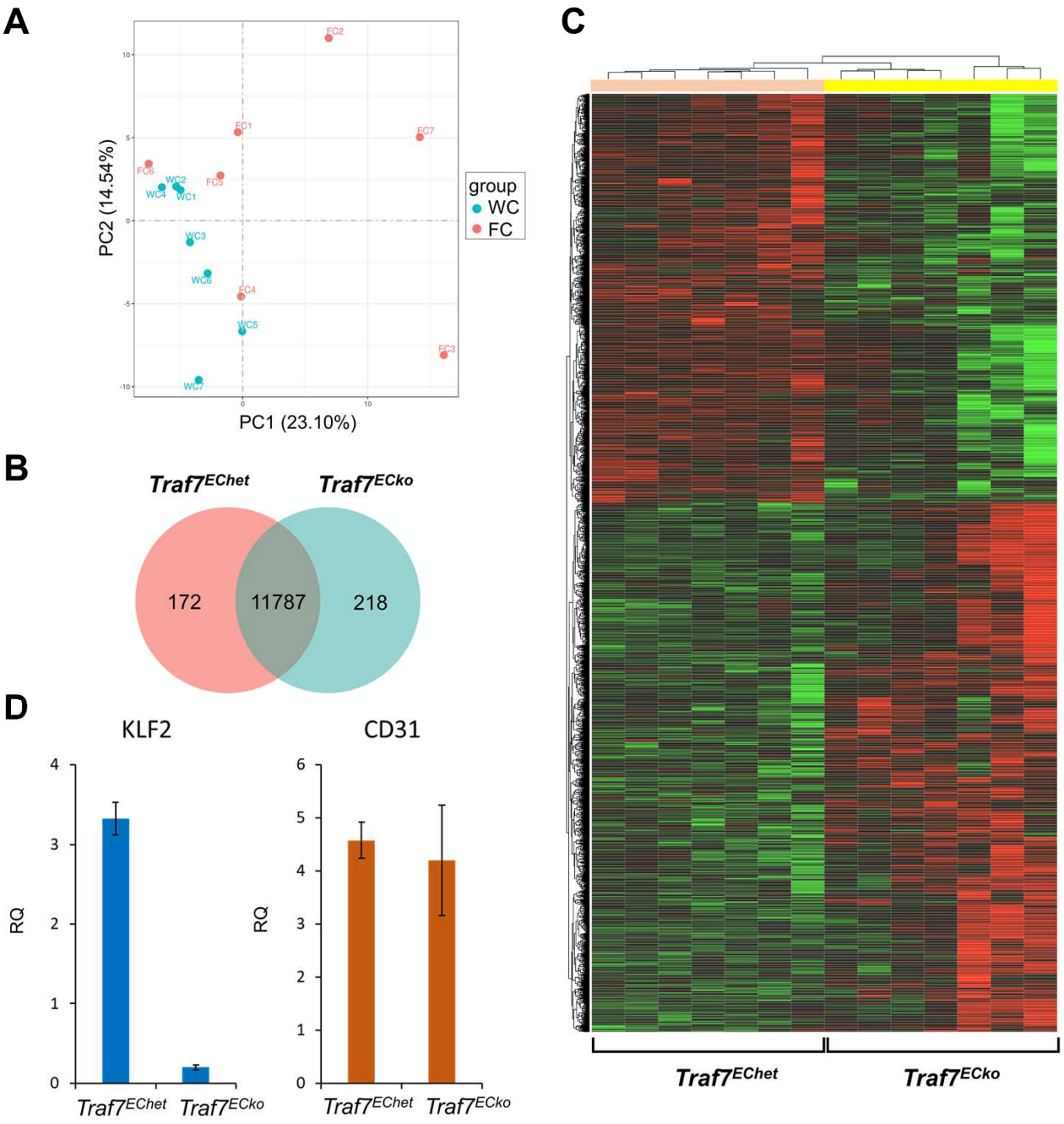
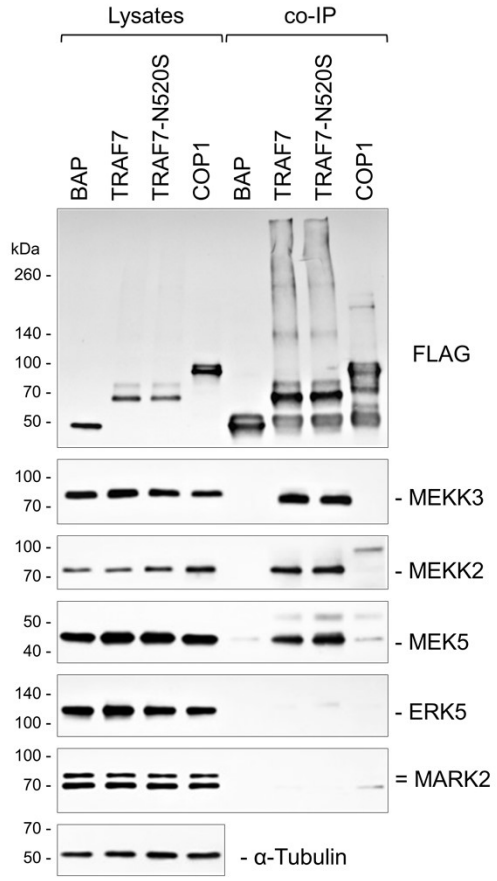


Figure S4. Altered gene expression in E9.5 embryos with endothelium-specific deletion of *Traf7*. (**A-C**) RNA-seq analysis of *Traf7*^{EChet} (WC) and *Traf7*^{ECko} (FC) embryos. (**A**) PCA Plot. Each point corresponds to an individual embryo. (**B**) Venn diagram showing DEGs overlap. (**C**) Heatmap of mRNA expression levels for all significant DEGs. (**D**) RT-qPCR mRNA expression analysis of *Klf2* and *Cd31* in WC (n=8) versus FC (n=7) embryos. *Gapdh* was used as an internal control. Data are presented as relative quantity (RQ) Mean ± SEM. Related to Figure 4D and E.

A



B

gene symbol	name	mascot score	protein mw	peptide match	sequence match
SCRIB	protein scribble homolog isoform a [Homo sapiens]	1759	178585	40	32
VBP1	prefoldin subunit 3 [Homo sapiens]	408	22783	11	8
NUDC	nuclear migration protein nudC [Homo sapiens]	274	38276	7	6
STUB1	E3 ubiquitin-protein ligase CHIP [Homo sapiens]	267	35290	7	6
PABPC1	polyadenylate-binding protein 1 [Homo sapiens]	241	70854	7	6
HNRNPA3	heterogeneous nuclear ribonucleoprotein A3 [Homo sapiens]	232	39799	11	6
HNRNPA0	heterogeneous nuclear ribonucleoprotein A0 [Homo sapiens]	220	30993	11	5
SNRNP200	U5 small nuclear ribonucleoprotein 200 kDa helicase [Homo sapiens]	216	246006	5	5

Figure S5. Association of TRAF7 with SCRIB.
(**A**) Western blot analysis of expression of several proteins and their co-immunoprecipitation (co-IP) with overexpressed FLAG-TRAF7 proteins using HEK293 whole cell lysates and indicated antibodies. Co-IP was done with anti-FLAG antibody. Anti-α-Tubulin antibody was used as the loading control. Blots shown are representative of at least four independent experiments. (**B**) Table showing top eight molecules associated with overexpressed TRAF7 in HEK293 by mass spectrometry analysis. Related to Figure 6.

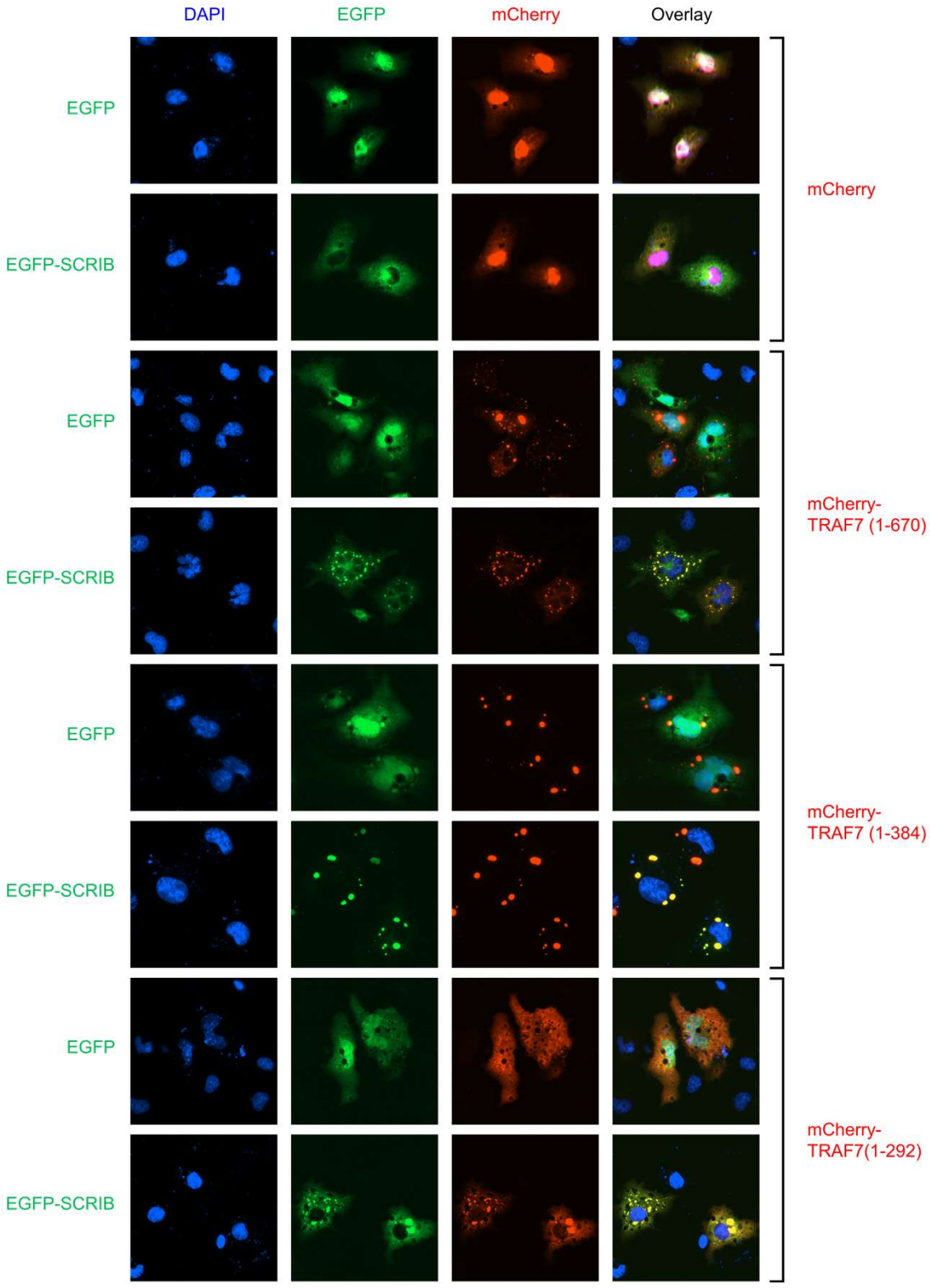


Figure S6. Association of TRAF7 with SCRIB.
Transient co-transfections of plasmids encoding SCRIB and full or truncated forms of TRAF7 as fluorescent fusion proteins in COS-1 cells line as indicated. Related to Figure 6.