Supplemental information

Ablation of collagen XII disturbs

joint extracellular matrix organization

and causes patellar subluxation

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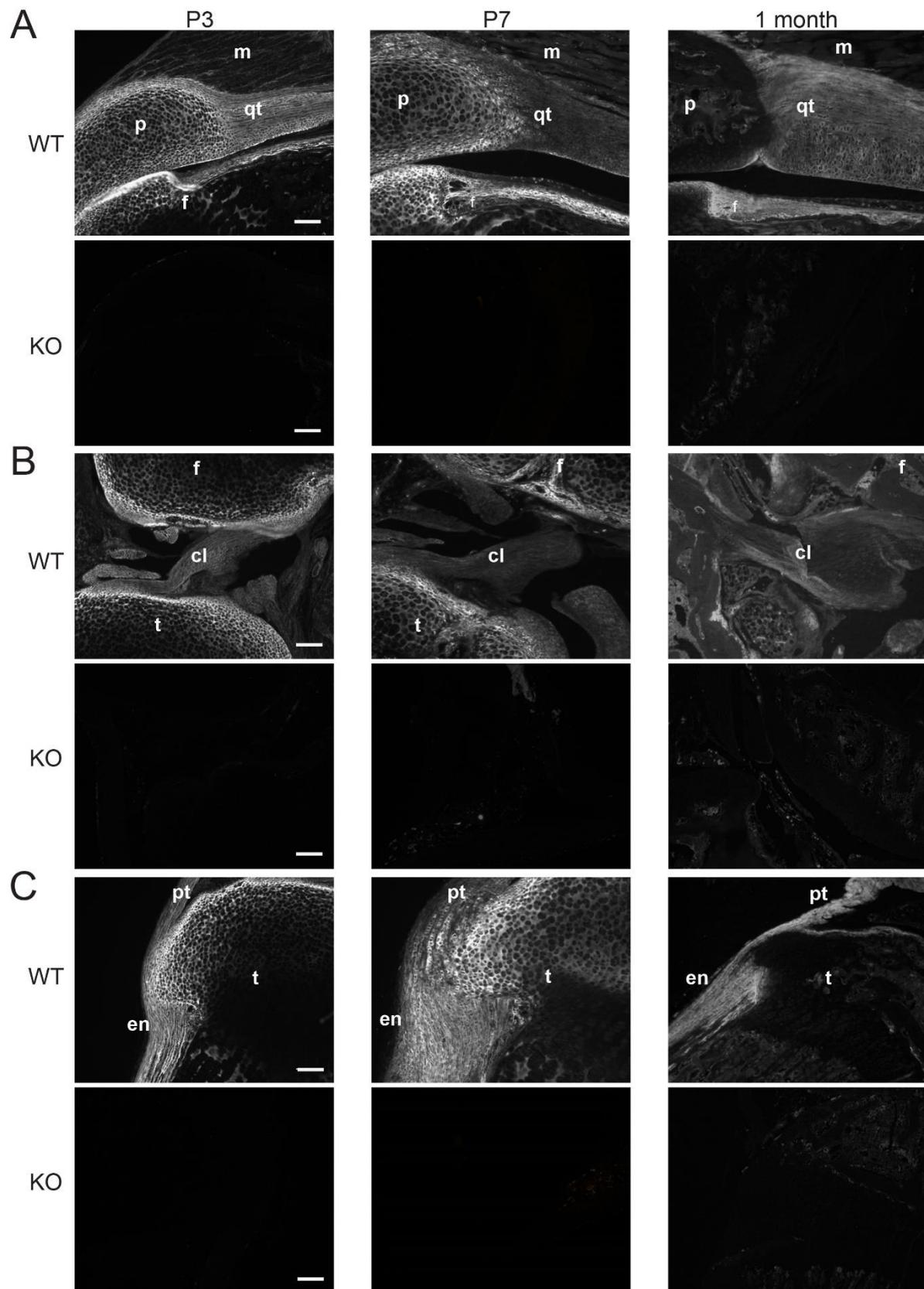
Figure S1. Immunofluorecent staining in hindlimb knee joint sections of WT and *Col12a1* KO mice, related to Figure 1. (A-C) Immunofluorescent staining of collagen XII in the whole hindlimb knee joints of P3, P7 and 1 month old WT and *Col12a1* KO mice. Patella (p), patella tendon (pt), femur (f), tibia (t) cartilage, cruciate ligament (cl), quadriceps tendon (qt), enthesis (en), and muscle (m) are shown in the pictures. Scale bar: 100 µm
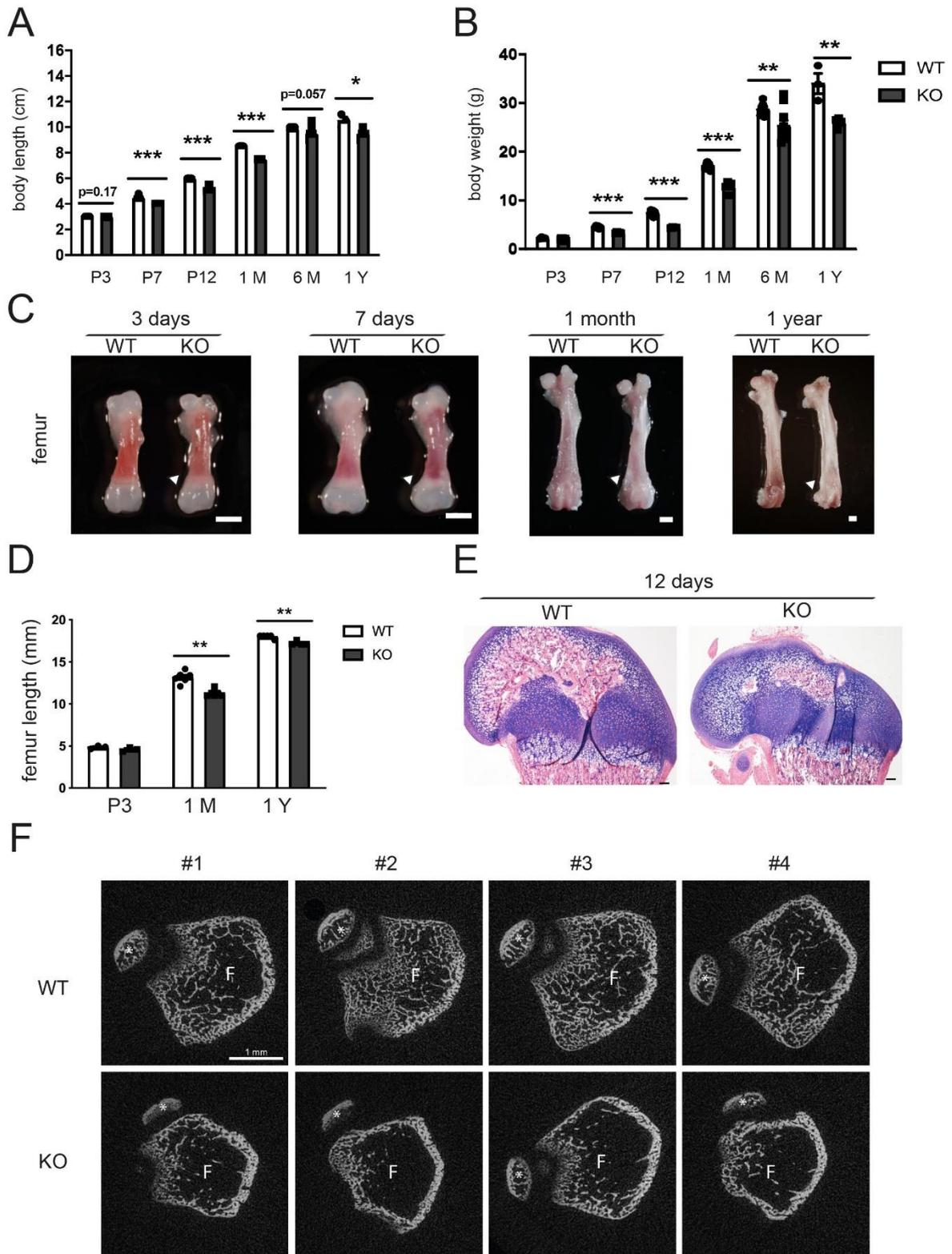
Figure S2. *Col12a1* KO mice are growth retarded, related to Figure 2. (A-B) *Col12a1* KO mice have a reduced body length and weight compared to their wildtype littermates at the age of postnatal 3 days (P3, n= 4 WT group, n= 9 KO group), postnatal 7 days (P7, n= 6 WT group, n= 5 KO group), postnatal 12 days (P12, n= 9 per group), 1 month (1 M, n= 7 WT group, n= 5 KO group), 6 months (6 M, n= 10 per group), and 1 year (1 Y, n= 5 per group). Unpaired two-tailed Student´s t-test, *, p < 0.05; **, p < 0.01; ***, p < 0.001. Error bars are mean ± SD. **(C)** Bending femoral ends were found in *Col12a1* KO mice (arrow heads) at the age of postnatal 3 days, 7 days, 1 month and 1 year. Scale

bar: 1mm. **(D)** Analysis of femur length in P3 (n= 3 per group), 1 month (n= 6 WT group, n= 4 KO group) and 1 year old mice(n= 5 WT group, n= 4 KO group). Unpaired two-tailed Student´s t-test, *, p < 0.05; **, p < 0.01. Error bars are mean ± SD. **(E)** H&E and alcian Blue staining - the secondary ossification centre formation was delayed at postnatal 12 days in *Col12a1* KO mice compared to control. Scale bar: 100 μm. **(F)** μCT analysis of 1 month old WT and *Col12a1* KO mouse joints (n=4 per group). The position of femur (F) and patella (asterisk) is indicated. Scale bar: 1 mm
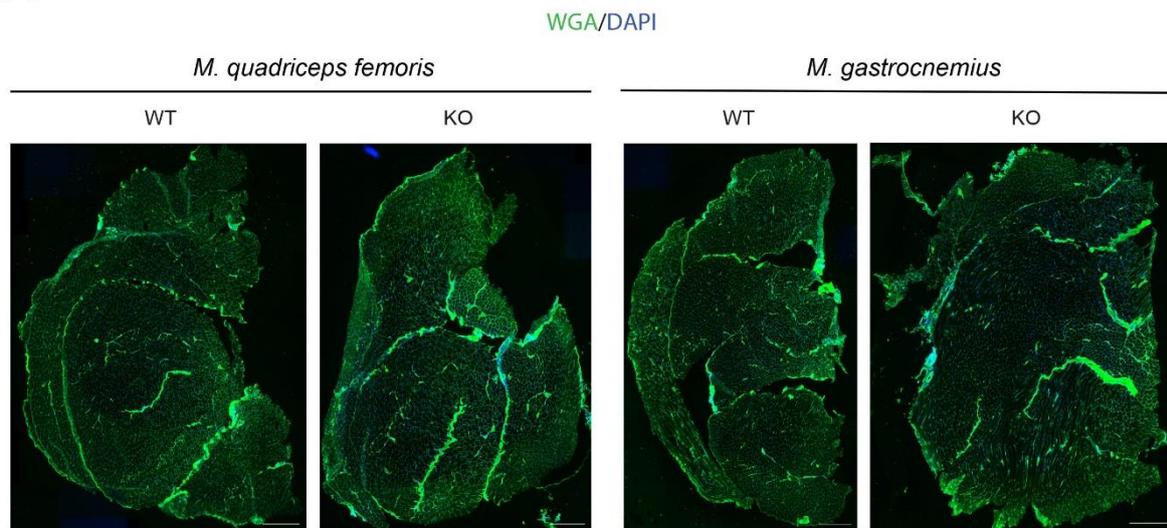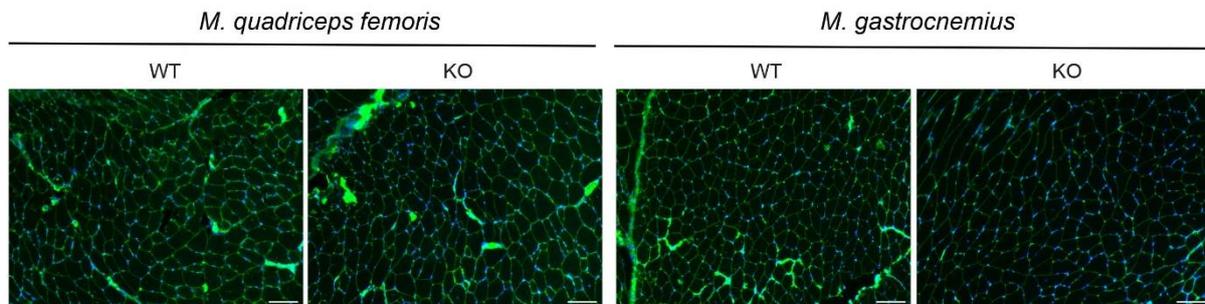
A

B

Figure S3. Immunofluorescent staining of *M. quadriceps femoris* **and** *M. gastrocenemius* **muscles, related to Figure 3. (A)** Representative images of WGA (green) and DAPI (blue) staining of *sections from the M. quadriceps femoris* and *M. gastrocnemius* muscles of WT and *Col12a1* KO female mice at the age of 6 months (n= 10 per group). Scale bar 500 μm. **(B)** Close ups, scale bar: 100 μm.
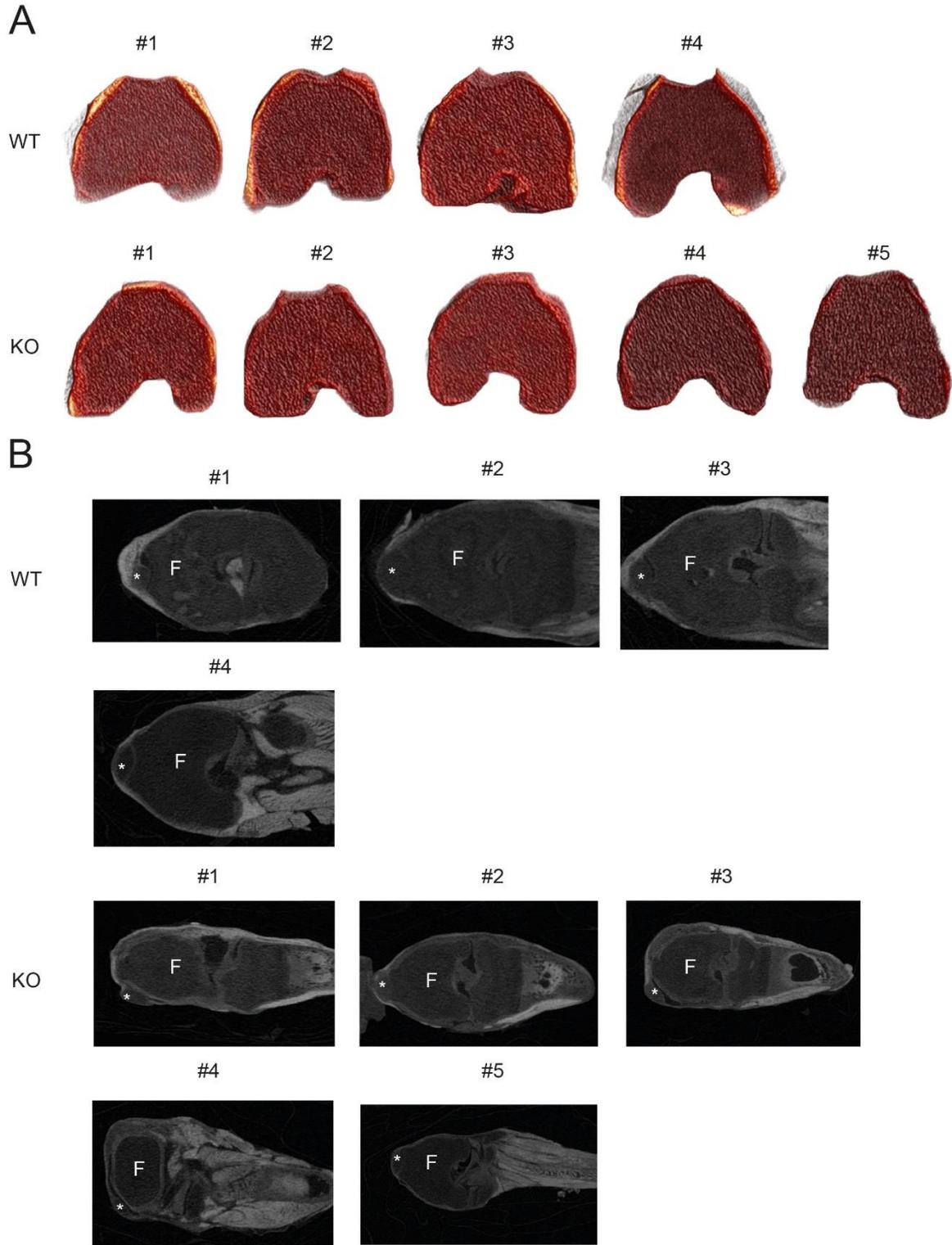
Figure S4. µCT volume renderings of femoral trochlear groove and patellofemoral alignment in P7 mice, related to Figure 4. (A) Images of µCT volume renderings of femoral trochlear groove from µCT analysis of P7 WT and *Col12a1* KO mouse joints (n= 4 WT group, n= 5 KO group). **(B)** Images of µCT analysis in P7 WT and *Col12a1* KO mouse joints (n= 4 WT group, n= 5 KO group). The position of femur (F) and patella (asterisk) is indicated.

PRID6028_agg

Count Summary   Count Gene Expression

24,203
Estimated Number of Cells

Aggregation ⓘ

Pre-Normalization Total Number of Reads	1,152,318,314
Post-Normalization Total Number of Reads	1,129,066,879
Pre-Normalization Mean Reads per Cell	47,611
Post-Normalization Mean Reads per Cell	46,650
Fraction of Reads Kept (SID149458)	95.9%
Fraction of Reads Kept (SID149457)	100.0%
Pre-Normalization Total Reads per Cell (SID149458)	47,697
Pre-Normalization Total Reads per Cell (SID149457)	47,527
Pre-Normalization Confidently Mapped Barcoded Reads per Cell (SID149458)	30,784
Pre-Normalization Confidently Mapped Barcoded Reads per Cell (SID149457)	29,524

t-SNE Projection Colored by Library ID

- SID149458
- SID149457
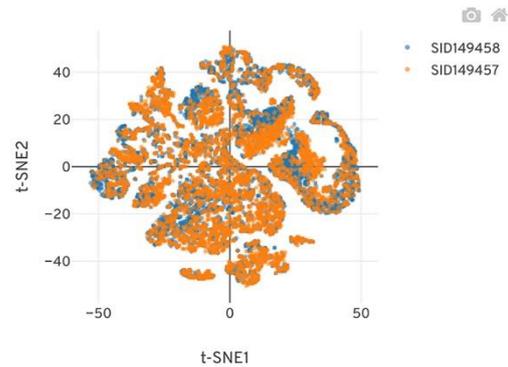
Cells ⓘ

Estimated Number of Cells	24,203
Fraction Reads in Cells	89.7%
Median UMI Counts per Cell	7,288
Median Genes per Cell	1,484

Sample

Sample ID	PRID6028_agg
Sample Description	
Chemistry	Single Cell 3' v3
Transcriptome	mm10
Pipeline Version	cellranger-7.0.0

Figure S5. Quality assessment of single cell RNA sequencing analysis, related to Figure 5. The quality assessment summary of the combined data set of two independent single cell RNA sequencing experiments is shown.