Osteopontin attenuates aging-associated phenotypes of hematopoietic stem cells

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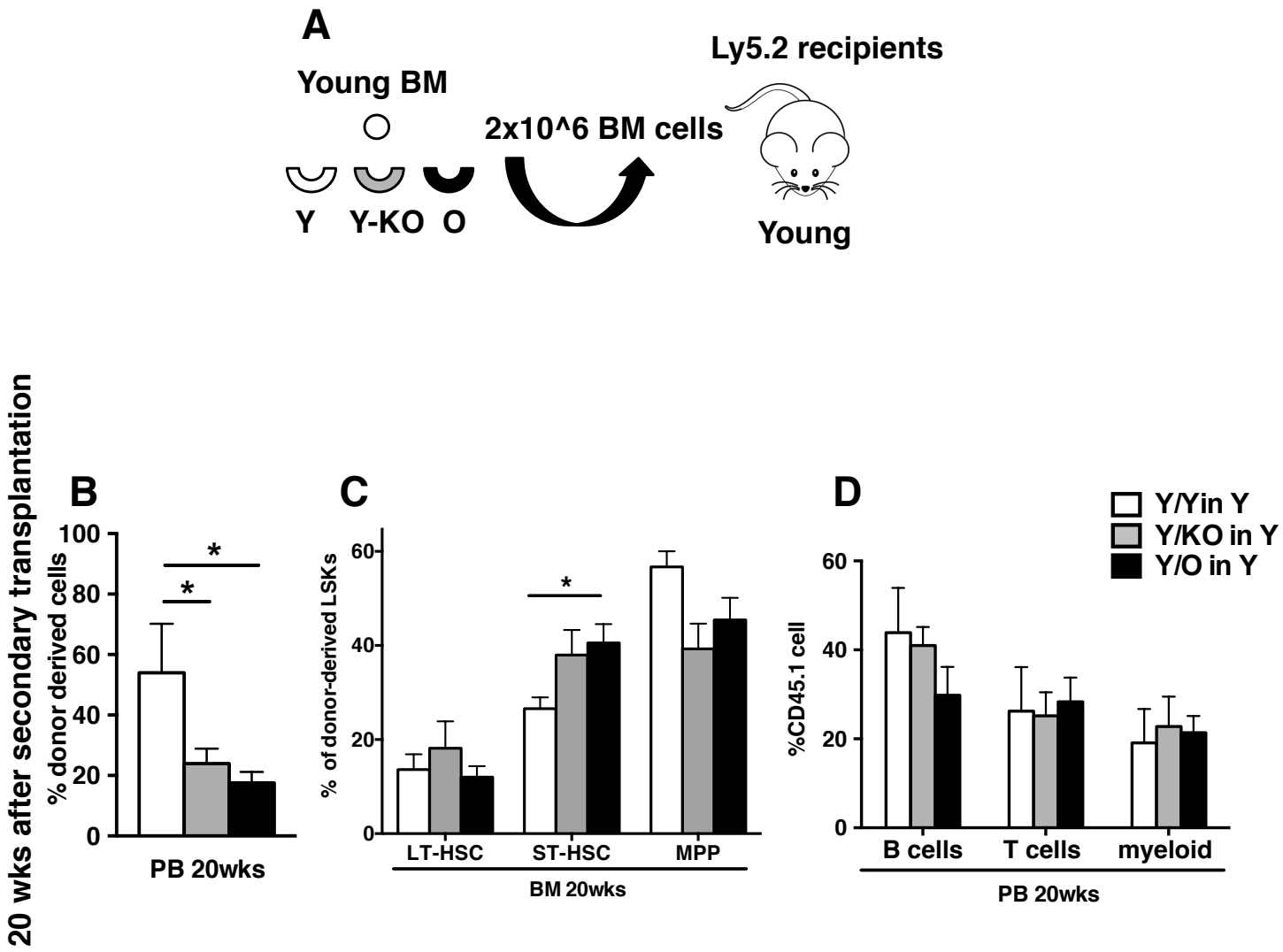
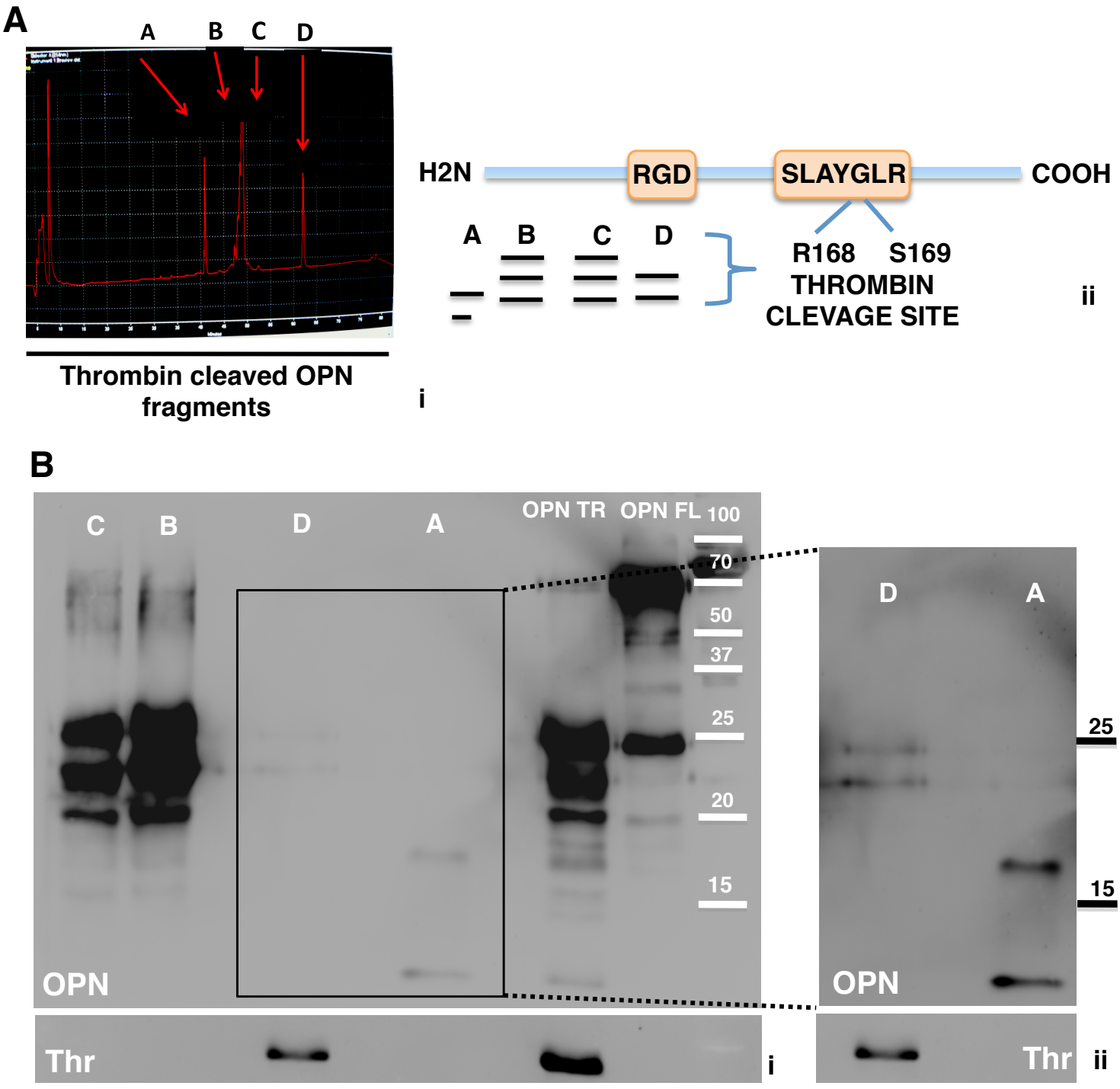
Appendix

Table of contents:



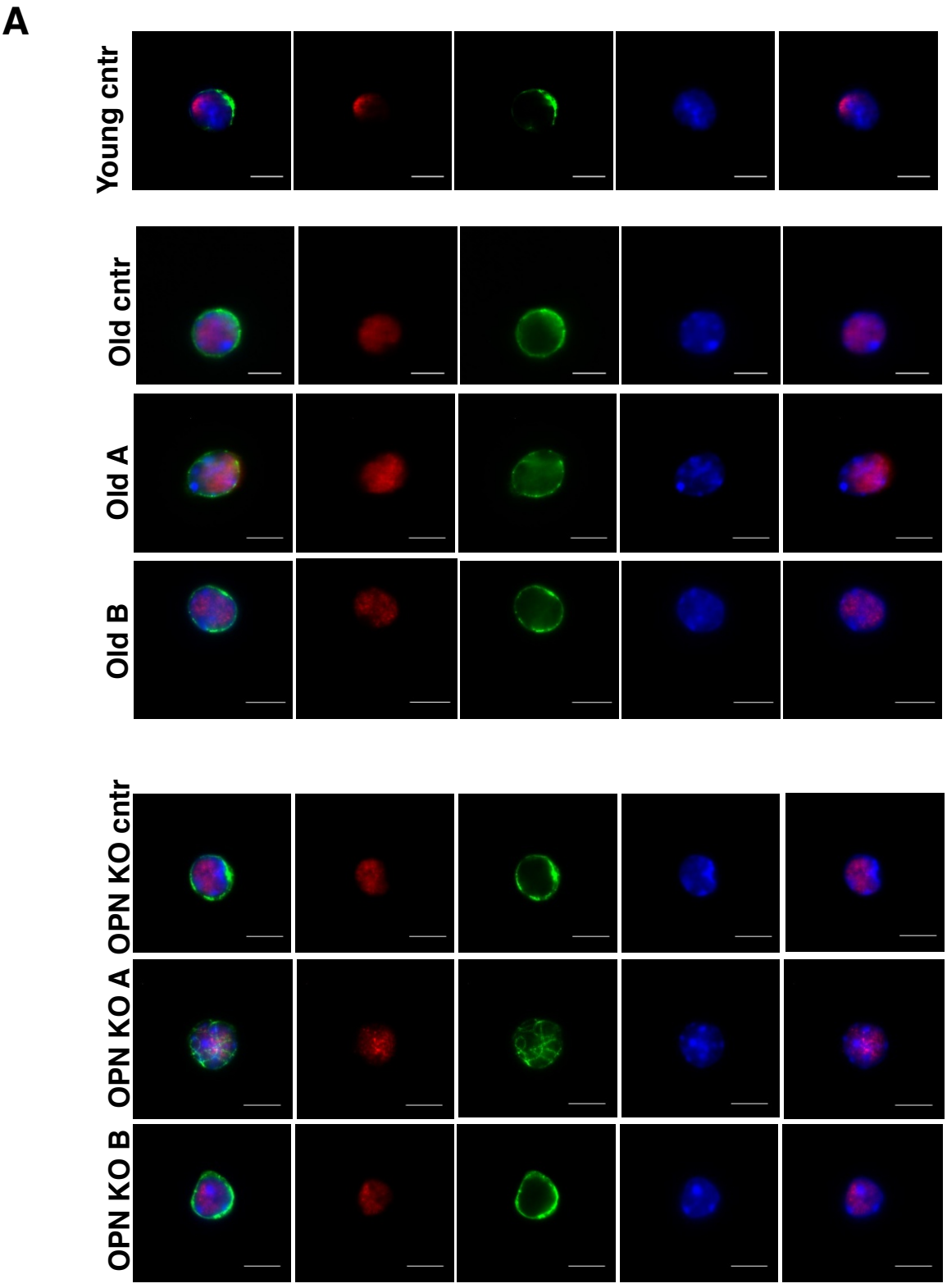
Appendix Figure S1. **Aging associated phenotypes of LT-HSC are attenuated upon secondary transplantation in young mice (A)** Schematic representation of the experimental setup **(B)** Frequency of young donor contribution (Ly5.1+ cells) to total WBC in PB in young recipients (Ly5.2+) mice. **(C)** Frequency of young LT-HSC, ST-HSC, and MPP cells in BM among donor-derived LSKs cells in young recipients (Ly5.2+) mice. **(D)** Frequency of young B cells (B220+), T cells (CD3+), and myeloid cells among donor-derived Ly5.1+ cells in PB in young recipients (Ly5.2+) mice (n=4-10 mice per group). Shown are means values + 1 SEM, *p<0.05.

Appendix Figure S1.

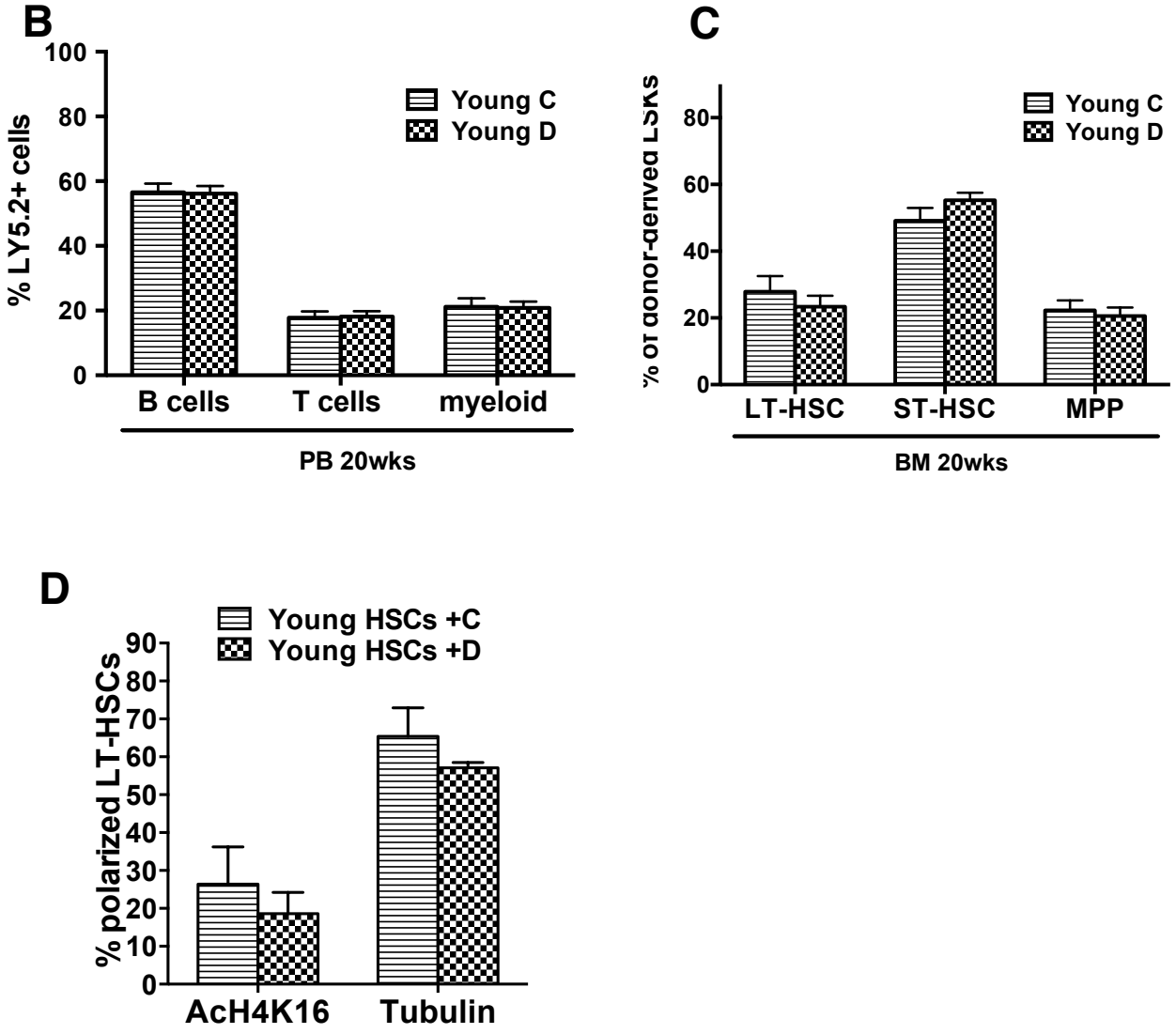


Appendix Figure S2. **OPN-cleaved fragments characterization. (A) (i)** Representative chromatogram of the thrombin digested OPN. n=3. Picks represent the main digested fragments. **(ii**) Schematic representation of osteopontin structure showing the generated fragments by a specific Thrombin clevage: 4 main fragments with different size were generated **(B) (i)** Western blot analysis was performed for all the OPN fractions generated to evaluate their molecular size compare to control samples OPN FL and OPN TR. **(ii)** Representative bands for fraction D and A.

Appendix Figure S2.



Appendix Figure S3.



Appendix Figure S3. **OPN fraction A and B does not re-polarize old and OPN KO LT-HSCs and ex-vivo treatment with OPN fraction C and D does not alter young LT-HSC function upon transplantation. (A)** Representative distribution of AcH4k16 (red) and tubulin (green) in old, old treated with fraction A, and old treated with fraction B LT-HSCs (same treatment condition for OPN KO LT-HSCs). Nuclei are stained with DAPI (blue). Bar=5 μm. **(B)** Frequency of young, young with fraction C and young with fraction D, B cells (B220+), T cells (CD3+) and myeloid cells among donor-derived Ly5.2+ cells in PB in young recipients (Ly5.1+) mice. **(C)** Frequency of young, young with fraction C and young with fraction D LT-HSC, ST-HSC and MPP cells in BM among donor-derived LSKs cells in young recipients (Ly5.1+) mice. **(D)** Percentage of LT-HSCs polarized for AcH4K16 and tubulin in donor-derived LT-HSCs (Ly5.2+ cells) sorted from the young, young with fraction C and young with fraction D experimental groups 20 weeks after transplant. ~40 cells scored per sample in each experimental repetitions (n =3).

Appendix Figure S3.