Osteopontin attenuates aging-associated phenotypes of hematopoietic stem cells

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Appendix

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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 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 cleavage: 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 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).