

SUPPLEMENTAL MATERIALS

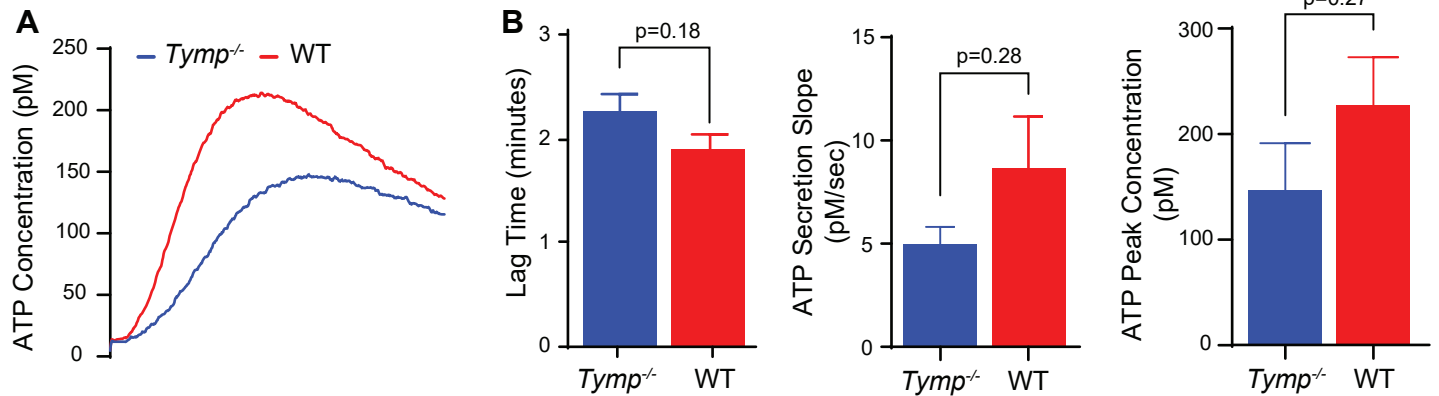
Targeting Thymidine Phosphorylase with Tipiracil Hydrochloride Attenuates Thrombosis without Increasing Risk of Bleeding in Mice

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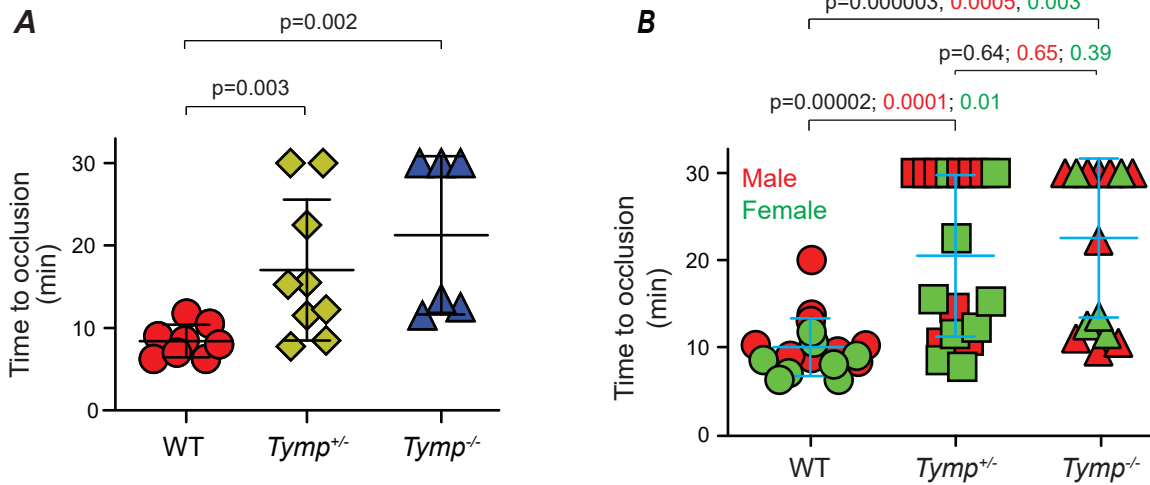
Supplemental Figure I



Supplementary Figure I. TYMP deficiency does not inhibit dense granule release. **A.**

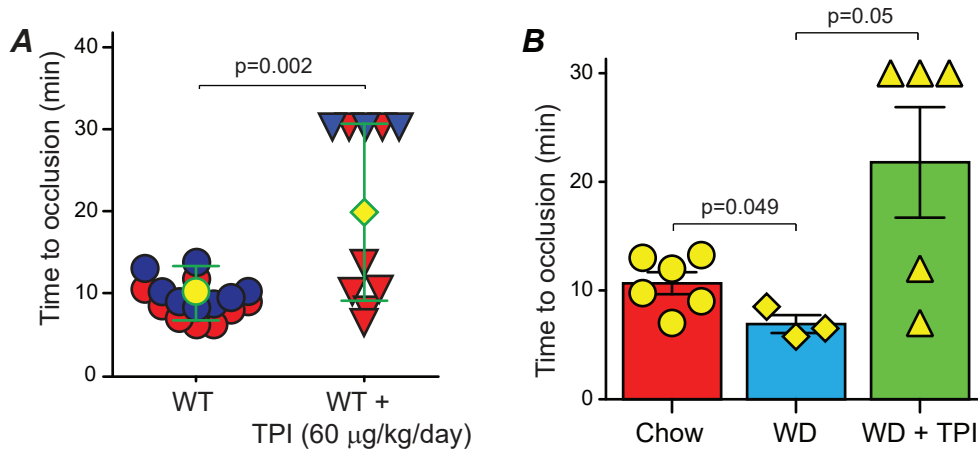
Platelet-rich plasma isolated from *Tymp*^{-/-} and wildtype (WT) female mice was stimulated with 1 $\mu\text{g}/\text{mL}$ collagen and ATP secretion was recorded. ATP release trace is representative of at least 5 mice. **B.** Lag time, ATP secretion slope, and ATP peak concentration were calculated based upon ATP release trace recordings. Bar graphs are shown as mean \pm SEM.

Supplemental figure II



Supplemental figure II. TYMP deficiency in mice inhibits thrombosis. 8-10 weeks WT, *Tymp*^{+/-}, and *Tymp*^{-/-} mice in both sexes were subjected to the 7.5% FeCl₃-induced thrombosis model. **A.** Time to occlusion in female mice. **B.** Time to occlusion in both male and female animals. *p* values in black font show the mixed sexes.

Supplemental Figure III



Supplemental Figure III. Oral administration of TPI inhibits thrombosis under both normal and hyperlipidemia conditions. **A.** WT mice were treated with TPI by gavage feeding for three days and then subjected to the FeCl₃ induced thrombosis model. **B.** WT mice fed with a western diet (WD, TD.88137) for 3 weeks were randomly divided into two groups. One group continually received WD, and another group received a customized WD (TD.190501) that gave mice TPI in a dose of 1 mg/kg/day for additional one week. Mice were then subjected to the thrombosis model and compared thrombosis in age-matched WT mice on chow.

Major Resources Table

In order to allow validation and replication of experiments, all essential research materials listed in the Methods should be included in the Major Resources Table below. Authors are encouraged to use public repositories for protocols, data, code, and other materials and provide persistent identifiers and/or links to repositories when available. Authors may add or delete rows as needed.

Animals (in vivo studies)

| Species | Vendor or Source | Background Strain | Sex | Persistent ID / URL |
|-----------------|------------------------|-------------------|-----|---|
| Mouse-Wild type | The Jackson Laboratory | C57BL/6J | M/F | https://www.jax.org/strain/000664 |
| | | | M/F | |
| | | | | |

Genetically Modified Animals

| | Species | Vendor or Source | Background Strain | Other Information | Persistent ID / URL |
|------------------------|-----------------------------------|--|---|--|---------------------|
| Parent - Male | Mouse- <i>Tymp</i> ^{-/-} | Dr. Michio Hirano Lab at Columbia University | C57BL/6J, has been back crossed for more than 10 times. | Initially, the male <i>Tymp</i> knockout mouse strain was a gift of Dr. Hirano. The male was mated with a female wild type C57BL/6J purchased from The Jackson Laboratory to generate the <i>TYMO</i> knockout mice. Currently, this mouse strain is maintained by homozygous breeding in Dr. Li' lab. | |
| Parent - Female | | | | | |

Antibodies

| Target antigen | Vendor or Source | Catalog # | Working concentration | Lot # (preferred but not required) | Persistent ID / URL |
|---------------------------|---------------------------|-----------|-----------------------|------------------------------------|---|
| Ser473-phosphorylated AKT | Cell signaling Technology | 4060S | 1:1000 | | Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb #4060/ https://www.cellsignal.com/products/primary-antibodies/phospho-akt-ser473-d9e-xp-rabbit-mab/4060?site-search-type=Products |
| Pan-AKT | Cell signaling Technology | 2920S | 1:2000 | | Akt (pan) (40D4) Mouse mAb #2920/ https://www.cellsignal.com/products/primary-antibodies/akt-pan-40d4-mouse-mab/2920?site-search-type=Products&N=4294956287&Ntt= |

DOI [to be added]

| | | | | | |
|------------|---------------------------|----------|--------------|--|---|
| | | | | | 2920s&fromPage=plp&_requestid=2133927 |
| TYMP | Cell Signaling Technology | 4307S | 1:1000 | | https://www.cellsignal.com/products/primary-antibodies/thymidine-phosphorylase-ecgf1-d69b12-rabbit-mab/4307?Ntk=Products&site-search-type=Products&N=4294956287&Ntt=thymidine+phosphorylase&fromPage=plp |
| GST(26H1) | Cell Signaling Technology | 2624S | 1:1000 | | https://www.cellsignal.com/products/primary-antibodies/gst-26h1-mouse-mab/2624?Ntk=Products&site-search-type=Products&N=4294956287&Ntt=gst&fromPage=plp |
| Lyn | Santa Cruz Biotechnology | SC-7274 | 1:800 | | https://www.scbt.com/p/lyn-antibody-h-6?requestFrom=search |
| TYMP | Santa Cruz Biotechnology | SC-56584 | 1:1000 | | https://www.scbt.com/p/pd-ecgf-antibody-spm322 |
| Actin | Santa Cruz Biotechnology | SC-8432 | 1:5000-10000 | | https://www.scbt.com/p/actin-antibody-c-2?requestFrom=search |
| TYMP | Abcam | ab180783 | 1:800 | | https://www.abcam.com/thymidine-phosphorylase-antibody-n-terminal-ab180783.html |
| P-selectin | BD Biosciences | 553744 | 1:100 | | https://www.bdbiosciences.com/ds/pm/tds/553744.pdf |

DNA/cDNA Clones

| Clone Name | Sequence | Source / Repository | Persistent ID / URL |
|------------------------|----------------|---------------------------|---|
| pcDNA6B/hTYMP | NM_001113755.3 | Original from Roche Japan | https://doi.org/10.1152/ajpheart.00176.2004 |
| pEGFP-N1-human lyn-GFP | | addgene #35985 | https://www.addgene.org/35958/ |
| pEBG | | Addgene #22227 | https://www.addgene.org/17741/ |

Cultured Cells

| Name | Vendor or Source | Sex (F, M, or unknown) | Persistent ID / URL |
|-------|------------------|------------------------|---|
| Cos-7 | AATC | | https://www.atcc.org/Products/All/CRL-1651 |
| | | | |
| | | | |

Data & Code Availability

| Description | Source / Repository | Persistent ID / URL |
|-------------|---------------------|---------------------|
| | | |
| | | |
| | | |

Other

DOI [to be added]

| Description | Source / Repository | Persistent ID / URL |
|--------------------|---------------------|---|
| Iron(III) chloride | 157740-100G | https://www.sigmaaldrich.com/catalog/product/sigald/157740?lang=en&region=US |
| Collagen | P/N385 | http://www.chronolog.com/Reagents.htm |
| ADP | P/N384 | http://www.chronolog.com/Reagents.htm |
| Chrono-Lume | P/N 385 | http://www.chronolog.com/Reagents.htm |