**Diverse Transformers (Trf) protein family in the sea urchin *Paracentrotus lividus* act through collaboration between cellular and humoral immune effector arms**

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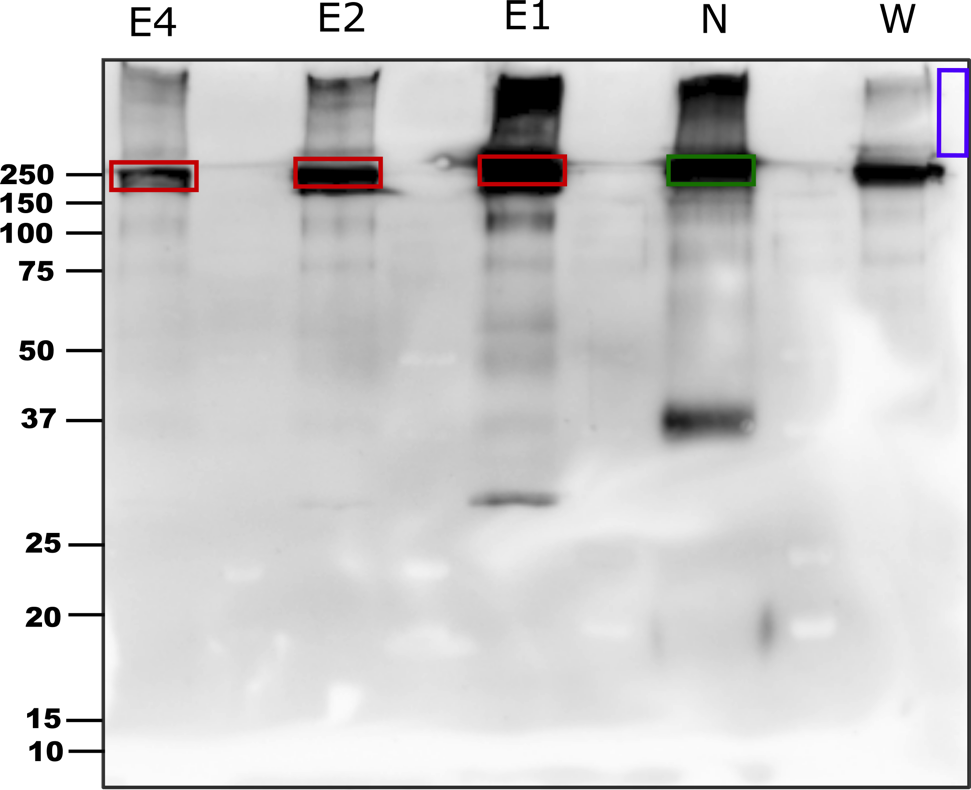
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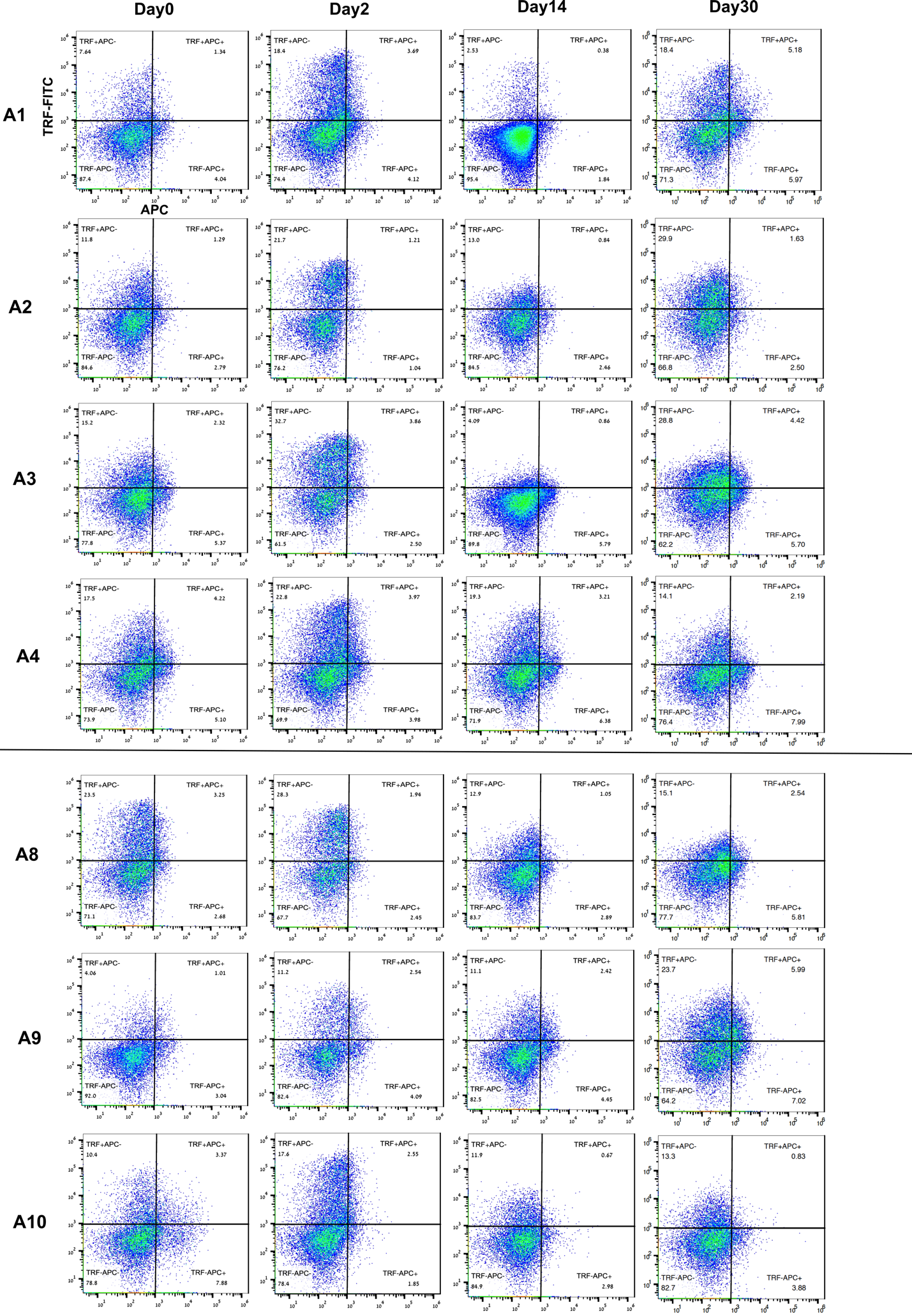
**Supplementary material**

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**Fig. S1 Unique Trf sequences obtained from publicly available databases.**

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**Fig. S2 Western blot of the gel that was used for the mass spectrometry.** E1, E2, E4 – elution fractions, N – untreated native whole coelomic fluid protein, W – wash fraction. Inside boxes are the bands that were cut from PAGE gel. In red – elution fractions, in green – native protein from the same animal, in blue – the background control that was cut from the side of the gel.

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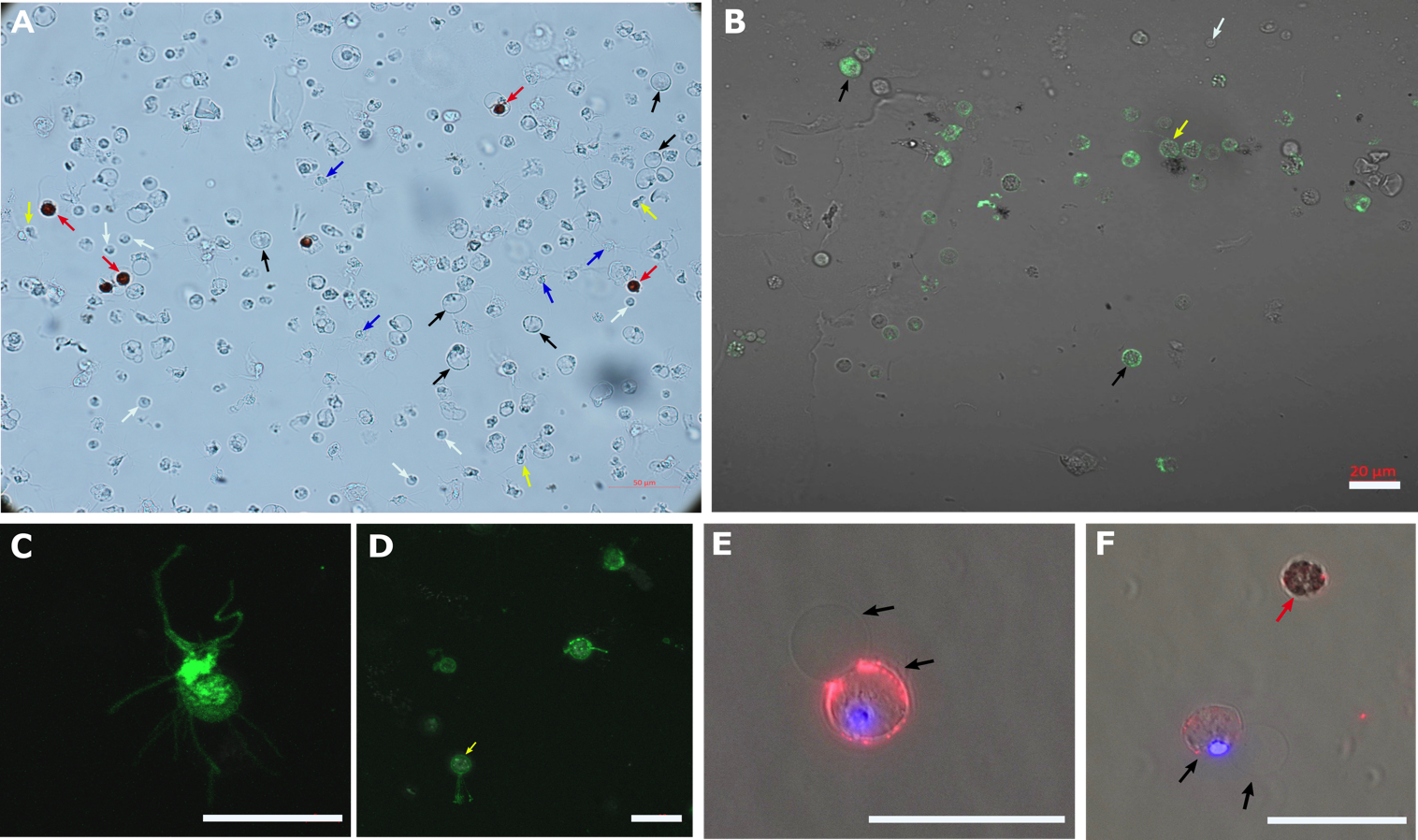
**Fig. S3 Trf-positive cell population change over time after injection of either *V. penaeicida* or Acf.** A1-A4 – animals challenged with heat-killed *V. penaeicida*, A8-A10 - control animals injected with sterile aCF. Y-axis Trf labeled with AF488, X-axis autofluorescence in APC channel indicates red spherule cells.

**Table S1 accessions of the Trf sequences used to build the phylogenetic tree.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| *H. erythrogramma\** | | *S. purpuratus\*s* | | *P. lividus\*\** | |
| JX245053.1 | HE001 | KJ408453.1 | SP001 | HACU01465650.1 | PL001 |
| JQ780274.1 | HE002 | KJ408451.1 | SP002 | HACU01465648.1 | PL002 |
| JX245045.1 | HE003 | KJ408450.1 | SP003 | HACU01465646.1 | PL003 |
| JQ780273.1 | HE004 | KJ408449.1 | SP004 | HACU01465645.1 | PL004 |
| JX245024.1 | HE005 | EF607775.1 | SP005 | HACU01465644.1 | PL005 |
| JX245021.1 | HE006 | EF607759.1 | SP006 | HACU01465643.1 | PL006 |
| JQ780304.1 | HE007 | EF607755.1 | SP007 | GFRN01311981.1 | PL007 |
| JX245020.1 | HE008 | EF607753.1 | SP008 | GFRN01311979.1 | PL008 |
| JQ780312.1 | HE009 | EF607742.1 | SP009 | GFRN01311974.1 | PL009 |
| JQ780311.1 | HE010 | EF607719.1 | SP010 | GFRN01311972.1 | PL010 |
| JQ780305.1 | HE011 | EF607717.1 | SP011 | GEDS01012457.1 | PL011 |
| JQ780303.1 | HE012 | EF607713.1 | SP012 | GEDS01012456.1 | PL012 |
| JQ780287.1 | HE013 | EF066272.1 | SP013 | GEDS01012454.1 | PL013 |
| JQ780284.1 | HE014 | EF066267.1 | SP014 | GEDS01012453.1 | PL014 |
| JQ780276.1 | HE015 | EF066259.1 | SP015 | GEDS01012452.1 | PL015 |
| JQ780275.1 | HE016 | EF066253.1 | SP016 | GEDS01012451.1 | PL016 |
| JQ780271.1 | HE017 | EF066219.1 | SP017 | GEDS01012450.1 | PL017 |
| JQ780270.1 | HE018 | EF066159.1 | SP018 | GEDS01012448.1 | PL018 |
| JQ780269.1 | HE019 | EF065743.1 | SP019 | GEDS01012447.1 | PL019 |
| JQ780268.1 | HE020 | EF065742.1 | SP020 | GEDS01012446.1 | PL020 |
| JQ780266.1 | HE021 | EF065733.1 | SP021 | GEDS01012445.1 | PL021 |
| JQ780265.1 | HE022 | DQ183180.1 | SP022 | GEDS01012443.1 | PL022 |
| JQ780262.1 | HE023 | DQ183182.1 | SP023 | GCZS01069164.1 | PL023 |
| JQ780244.1 | HE024 | DQ183168.1 | SP024 | GCZS01069162.1 | PL024 |
| JQ780233.1 | HE025 | DQ183167.1 | SP025 | GCZS01069159.1 | PL025 |

*\* P. lividus*, *S. purpuratus* and *H. erythrogramma* Trf sequence accession numbers from NCBI protein database

*\*\*P. lividus* sequences from TSA NCBI databases (HACU01, GCZS01, GEDS01, GFRN01)



**Fig S4. *P. lividus* coelomocyte types and sorted Trf-positive coelomocytes.** **A.** *P. lividus* unsorted coelomocytes in whole CF shown in a bright field. **B.** Sorted Trf-positive cells - petaloid phagocytes and a vibratile cell labeling is clearly seen, other coelomocyte types have a weaker signal. **C.** Trf protein expression on the cell surface and filopodia of a filopodial phagocyte (confocal microscopy) **D.** Trf-positive *P. lividus* vibratile cell (yellow arrow) (confocal microscopy). **E and F.** Membranal interactions of Trf-positive and Trf-negative petaloid phagocytes (black arrows). Scale bars = 20 μm. Blue arrows - filopodial phagocytes. Red arrows- red spherule cells. Yellow arrows - vibratile cells. Green fluorophore — 2nd Ab AlexaFluor 488. Red fluorophore - 2nd Ab AlexaFluor 567. Blue is DAPI.